STRUCTURAL STUDIES ON ALGAL POLYSACCHARIDES

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Foreword

The thesis consists of reprints of published work carried out in the Chemistry Department of the University of Edinburgh. None of it has been submitted by the candidate for any other degree.

To the earlier papers, in collaboration with Dr. E.G.V.Percival, the writer made independent contributions and was responsible for the whole of the experimental work. In six of the remaining publications (10, 11, 19, 21, 24 and 26)* a considerable part of the experimental work was performed by the writer. In the other papers the practical work was carried out by various students during the course of training for the degree of Ph.D., but in all cases the researches were initiated by the writer and carried out under her direction.

Those papers in which a senior author's name occurs form part of the general programme of carbohydrate research in the Department of Chemistry of the University of Edinburgh.

The one paper (31) which is submitted in manuscript form has been accepted for publication in the Journal of the Chemical Society.

*Figures in parenthesis refer to the list of papers in the thesis.

STRUCTURAL STUDIES ON ALGAL POLYSACCHARIDES

The algae may be divided into four groups according to their colour: the Phaeophyceae (brown algae), the Rhodophyceae (red algae), the Chlorophyceae (green algae) and the Cyanophyceae (blue green algae). Very little is known about the metabolism of the blue green algae, but the other groups of seaweeds synthesise characteristic polysaccharides. The major constituents of the brown algae are the polyuronide, alginic acid, the 1,3'-β-linked glucan, laminarin, and the sulphated fucose containing polymer, fucoidin. The red algae on the other hand mainly synthesise sulphated 1,3'-linked D-galactopyranose containing polysaccharides, some of which may also contain 3,6-anhydro-L-galactose residues. In addition a xylan has been separated from Rhodymenia palmata, and a mannan from Porphyra umbilicalis. In the field of the green algae the polysaccharide constituents are even more complex, aqueous, dilute acid, and alkaline extraction giving rise to a mixture of water-soluble sulphated polysaccharides comprising a variety of hexoses and pentoses and in some instances uronic acid residues. In contrast land plants do not appear to synthesise polysaccharides which carry sulphate groups, but the important animal polysaccharides, chontroitin sulphates,

of cartilaginous tissue, and the blood anticoagulant, heparin, are all sulphated.

The polysaccharides from the green seaweeds have many properties in common with the sulphated animal polysaccharides. They are very difficult to purify and to separate from protein. Uronic acid residues appear to occur in both groups of polysaccharides, but whereas the animal polysaccharides all contain amino sugar residues, so far this class of sugar has not been detected in the green seaweed polysaccharides; the latter containing a relatively large proportion of xylose, arabinose and rhamnose units.

The sulphate radicals in algal polysaccharides are present as sulphate ester groups, $ROSO_3^-$, they are hydrolysed by acids and no sulphate ions can be detected in a solution of a sulphated polysaccharide until the sulphate group has been cleaved. They usually occur as mixtures of salts of several different metals such as sodium, potassium, magnesium and calcium.

There are a wide variety of chemical methods which can be applied to the elucidation of polysaccharide structure. In the first instance, hydrolysis followed by the separation and characterisation of the hydrolysis fragments establishes the sugar units present in the polysaccharide.

Similar treatment of the methylated polysaccharide determines which hydroxyl groups in the sugar residue are involved in the glycosidic linkages.

Diethyl mercaptan in the presence of concentrated hydrochloric acid causes scission of the glycosidic links in a polysaccharide with the formation of the diethyl mercaptal derivatives of the sugars released during the reaction. These derivatives are often stable crystalline compounds which are readily characterised. This method of attack has proved valuable for the detection of constituents such as 3,6-anhydrosugars (12) which are degraded during acid hydrolysis of the polysaccharide.

In heteropolysaccharides the isolation and characterisation of oligosaccharides from partial hydrolysates provides information of the relative position of the different sugar residues (20, 22, 29).

Periodate oxidation is also of value in structural investigations on polysaccharides. Sodium metaperiodate cleaves α-glycol groups with the reduction of one mole of periodate. Quantitative determination of the amount of formic acid and formaldehyde released from the sugar residues and the quantity of periodate reduced on oxidation of a polysaccharide, besides providing information on the main

types of linkage present, also enables calculation of the average chain length of the polysaccharide molecules. This method of investigation has the added advantage of requiring only milligram quantities of polysaccharide material.

Chromatographic examination of the acid hydrolysate of the residual polysaccharide (oxopolysaccharide) remaining after periodate oxidation reveals the presence of those sugar units which are unattacked by periodate (20, 23, 27, 28, 29). This is particularly useful in the determination of possible branching in a polysaccharide, since sugar units at the branch points may be linked through at least three positions and hence be immune to periodate oxidation.

Barry degradation, an extension of periodate oxidation, has proved of value in the elucidation of polysaccharide structure particularly in complex polysaccharides in which only the outer units are attacked by periodate. By heating the oxidised polysaccharide with phenylhydrazine under mildly acid conditions, the fragments of the sugar units which have been attacked by periodate at the non-reducing ends of the chains are removed from the polysaccharide and fresh sugar residues are exposed which are now susceptible to attack by periodate. By repeated

periodate oxidation and Barry degradation the outer chains of a highly branched polysaccharide may be removed, leaving a relatively simpler polymer for examination (23, 28). Information can also be gained from the separation and characterisation of the aldehydic fragments which are removed as osazones and phenyldrazones during the treatment with the phenylhydrazine.

Uronic acid residues in a polysaccharide are often difficult to identify. Relatively drastic conditions of acid hydrolysis are necessary for cleavage of the uronosyl linkages and the liberated uronic acid is frequently degraded by the hot acid as it is released. Partial hydrolysis, followed by reduction of the oligouronic acid fragments to neutral oligosaccharides has surmounted this difficulty in a number of cases (22, 24, 29).

Although the earlier investigations described in this thesis were completed prior to the application of chromatography to carbohydrate chemistry, its invaluable help in the later studies cannot be stressed too highly; qualitative and preparative separation on paper, preparative separation on columns of cellulose, silica gel, alumina and amberlite resins have all been utilised.

ALGAL POLYSACCHARIDES

The elucidation of the molecular structure of algal polysaccharides presents problems of particular difficulty; the mucilages are difficult to extract and purify without degradation of the polysaccharides, the presence of sulphate and uronic acid residues hinders methylation and often prevents complete methylation of the molecules. Furthermore, since the methylated products are soluble in the methylation reaction mixture and cannot be extracted in the usual manner, isolation is accomplished by dialysis after each methylation. The increased stability of the glycosidic link of the uronosyl units and the degradation of these residues during hydrolysis with acid reduces the information available from hydrolytic studies. Nevertheless, application of the different methods of structural investigation, coupled with model experiments on monosaccharides, has resulted in the elucidation of the main structural features of the water-soluble polysaccharides of the green seaweeds, Cladophora rupestris, Acrosiphonia centralis, and Caulerpa filiformis, and provided information on the finer details of the structure of the mucilage from Ulva lactuca, of carrageenin the red seaweed polysaccharide, and of laminarin and alginic acid from the brown algae.

Cladophora rupestris.

Aqueous extraction of Cladophora rupestris at pH 3-4 gave a complex mixture of sulphated polysaccharides (15, 20). This extract was contaminated with 20-25% of protein which could not be removed completely by any of the routine methods. Precipitation of protein with trichloracetic acid, followed by fractional precipitation of the polysaccharide with ethanol gave the purest product. The fraction precipitated with 95% ethanol had the lowest protein contamination ca 8%, and structural investigations were carried out on this material which had $[\alpha]_D$ +69° and contained sulphate 19.6%, ash 13.7, and arabinose, galactose, xylose, rhamnose and glucose in the ratio of 14:13:4:1.5:1. All attempts to fractionate it into more than a single polysaccharide were unsuccessful until after acetylation. Chloroform extraction of the acetate separated a sulphate-free glucose-rich fraction resembling the 1,3-linked β-glucan, laminarin. The residual material contained approximately two sulphate residues to every five anhydrosugar units and these were extremely stable to alkali even under conditions which degraded the polysaccharide.

Partial hydrolysis, methylation and periodate oxidation studies on this residual glucose-free material established

that some of the galactose and xylose occurred as non-reducing end groups, that the rest of the xylose was present either as end units carrying sulphate groups on C_4, or more probably as 1,4'-linked xylose units, and that 1,3'-linked L-arabopyranose, D-galactopyranose, L-rhamnopyranose and 1,6'-linked galactofuranose units were also present. The difficulty of preparing a fully methylated polysaccharide made an assessment of the part the lower methylated monosaccharides, isolated from the methylated hydrolysate, played in the overall structure of the polysaccharide impossible. Undoubtedly some of them carried sulphate groups since the methylated polysaccharide contained 16.2% sulphate.

Oligosaccharides separated from a partial acid hydrolysate provided evidence for the presence of contiguous arabinose units and of the presence of arabinose, xylose and galactose units in a single polysaccharide.

The application of the Barry degradation to the water-soluble extract from C.rupestris (28) confirmed the above results. Repeated successive treatment with periodate and phenylhydrazine gave a 25% yield of a sulphated degraded polymer containing L-arabinose, D-galactose and L-rhamnose in the molar proportions of 1:1:0.6. The sulphate content

of the oxo- and degraded polysaccharides isolated after each treatment remained approximately constant, indicating that these residues were not restricted to the periphery, but were spread evenly throughout the molecule(s).

Methylation of the degraded material yielded an almost completely methylated product with a methoxyl content of 27%. (Calculated methoxyl is approximately 28%). Separation and characterisation of the methylated sugars present in the hydrolysate of this material indicated that all three sugars were 1,3'-linked, that sulphate residues were probably located on C_4 of the arabinose units (see page 29), and that a high proportion of the units were triply linked.

Certain broad conclusions can be drawn concerning the main polysaccharide material extracted from C.rupestris. It has a highly branched structure with xylose and galactose units at the ends of the branches, and galactose, arabinose and rhamnose residues in the inner portion of the molecule. Sulphate groups are linked to residues both on the outer branches and in the centre of the molecule. Finally it may be mentioned that after three oxidations with periodate and two treatments with phenylhydrazine the molecule was still sufficiently large to be retained by a dialysis sac.

In conclusion it should be emphasised that, although the water-soluble extract from C.rupestris gave a Schlieren electrophoretic diagram with a symmetrical peak indicating a monodispersed polysaccharide, a final decision concerning the homogeneity of the mucilage cannot be reached with the techniques at present available.

Acrosiphonia centralis.

The water-soluble extract from Acrosiphonia centralis (Spongomorpha arcta) (25, 29) was devoid of arabinose residues and differed further from C.rupestris extract in its relatively low sulphate (7.8%) and high glucose and rhamnose contents, and in the presence of ca 20% uronic acid residues. Comparison of the constituents of this material with the water-soluble extract from the green seaweed, Ulva lactuca (Brading, Georg-Plant and Hardy, J., 1954, 319) shows a close similarity between the two materials; the major differences being the higher proportion of sulphate (17.5%) and rhamnose residues in the U.lactuca extract.

The polysaccharide material was extracted from A.centralis as the ammonium salt with 1% aqueous ammonium sulphate and after the removal of inorganic ions by dialysis was isolated as an off-white powder by freeze-drying. It

was contaminated with less than 4% protein and had a sulphate content of 7.8%, and contained xylose, rhamnose and glucose in the ratio of 1.6:1.4:1 with trace quantities of galactose and mannose. Preferential precipitation experiments failed to separate the material but chloroform extraction of the acetylated polysaccharides separated about 9% of a glucose-rich fraction which differed from the glucan separated from C. rupestris in that it was a 1,4'-linked α-glucan with branch points at C_6 and further resembled starch in its colour reactions with iodine.

Methylation studies on the residual sulphated polysaccharide(s) revealed the presence of 1,4'-linked xylose and rhamnose, end-group xylose and a relatively high proportion of triply linked rhamnose. All the glucuronic acid residues appeared to be present as end group linked to rhamnose. Periodate oxidation studies indicated a high proportion of cis glycol groups confirming the methylation results.

The sulphate groups were stable to alkali; this, coupled with the methylation results, points to their location on C_3 or C_4 of the rhamnose units. In 1,4-linked rhamnose, sulphate groups on C_3 or in 1,3'-linked rhamnose sulphate on C_4 would be alkali stable (see page 29).

Partial acid hydrolysis of the original extract from A.centralis led to the isolation and characterisation of the aldobiuronic acid, 4-O-β-D-glucuronosyl-L-rhamnose. This is the first time glucuronic acid linked to C_4 of rhamnose has been found in Nature. Other oligouronic acid fractions were separated, all of which contained rhamnose and glucuronic acid residues.

From the information available it is impossible to advance a unique structure for this sulphated mucilage; the evidence points to chains of 1,3'-linked rhamnose units carrying glucuronic acid residues linked at position 4. Whether the xylose residues are also linked to the rhamnose or whether the xylose is present as a separate 1,4'-linked xylan must await further experimental evidence.

Recent experiments on an aqueous extract of Ulva lactuca (J. McKinnell and Elizabeth Percival, unpublished work) have resulted in the separation of 4-O-β-glucuronosyl-L-rhamnose, the aldobiuronic acid isolated from the hydrolysate of a similar extract of A.centralis; this emphasises the similarity between the two species.

Caulerpa filiformis.

The green seaweed, Caulerpa filiformis, found on the coasts of S. Africa, again yielded a complex mixture of

polysaccharides on hot aqueous extraction (27, 31). In contrast to the extracts from the other green seaweeds, addition of cetyltrimethylammonium hydroxide (Cetavlon) to the borate complexes of the polysaccharides precipitated the sulphated material and left a sulphate-free glucan in the supernatant solution. Methylation, periodate oxidation, and enzymic studies on this glucan (31) revealed many similarities between it and the amylopectin fraction of starch.

The sulphated material recovered from the Cetavlon complex contained mainly galactose with lesser amounts of glucose, xylose, mannose, and rhamnose. A small quantity of uronic acid may also be present but this still awaits confirmation.

When the initial extraction of C. filiformis was carried out in the cold under nitrogen, arabinose units were also found to be present in the polysaccharides; these units apparently being lost by autohydrolysis during the hot extraction. Incubation of the extracts with salivary α-amylase followed by dialysis completely removed the glucose units, indicating that the glucose-containing material precipitated by the Cetavlon is probably the same as the amylopectin type material left in the supernatant. The small quantity of

residual sulphated polysaccharide (SO_4^{2-}, 17%) which is available (ca 1% of the dry weight of weed) has precluded extensive structural investigation. Mild acid hydrolysis followed by fractionation on Amberlite resin has given evidence of the presence of both sulphated and non-sulphated oligosaccharides. The stability of the sulphate groups to alkali and the resistance of the polysaccharide to oxidation with periodate indicates a high proportion of 1,3'-linked residues with sulphate groups on C_4 of the galactose units.

After aqueous extraction the residual C.filiformis weed was subjected to a mild chlorite treatment. Dilute alkaline extraction of the residual weed removed polysaccharide material consisting of 96% xylose and 4% glucose residues (27). After purification, a water-soluble polysaccharide containing only xylose units was isolated. Structural studies showed that this consisted of essentially linear chains of β-1,3'-linked D-xylopyranose units. Although 1,4'-linked xylans constitute the major polysaccharide of the hemicelluloses of land plants and a xylan containing 80% of 1,4'-linked and 20% of 1,3'-linked β-D-xylopyranose units has been separated from the red seaweed Rhodymenia palmata (Chanda and E.G.V. Percival, Nature, 1950, 166, 787), this is the first recorded instance of a xylan consisting solely of

1,3-linked D-xylose residues.

It may be mentioned that the glucose containing material separated in the purification of this xylan was different from the amylopectin-type material found in the aqueous extract. It gave no colour with iodine, reduced relatively little periodate and glucose units were present in the hydrolysate of the oxopolysaccharide. In addition crystalline 2,4,6-tri-O-methylglucose was separated from the methylated hydrolysate. All these results, together with rotational measurements, indicate a 1,3-linked β-glucan structure of the laminarin type for this polysaccharide.

All the green seaweeds, so far studied, synthesise galactose, xylose and rhamnose containing polysaccharides which carry varying proportions (6-21%) of sulphate groups. As much as 20% may also comprise uronic acid units, but in species of Cladophora and Caulerpa the presence of uronic acids has still to be established; if present at all they can only occur in very small quantities in these species of seaweed.

Determination of the position of linkage of the sulphate groups in these polysaccharides has proved difficult. If it were possible to remove the sulphate groups without affecting the glycosidic links, an examination

of the products of hydrolysis of the methylated desulphated polysaccharide in comparison with those from the original methylated substance, would decide the position of these groups.

The sulphate residues however all proved stable to alkaline hydrolysis and the strength of acid necessary for their removal also cleaved the glycosidic links of the polysaccharides. Consequently allocation of these residues to particular hydroxyl groups in the sugar units has only been possible from presumptive deductions arrived at from a study of monosaccharide toluene sulphonate derivatives (see pages 26-29)

The identity of the galactose polysaccharides of the green seaweeds with the sulphated galactans of the red algae awaits the separation of a pure galactan from the complex mixtures obtained on aqueous extraction of the former. All the evidence available, however, confirms the presence of a similar structure, namely, chains of 1,3'-linked galactose units with sulphate groups on C_4.

Although the xylan isolated from C.filiformis has been definitely established as a 1,3'-linked xylan, periodate oxidation and methylation studies on C.rupestris and A.centralis point to the presence of 1,4'-linked xylose

units in the polysaccharides isolated from these two species of algae.

The presence of starch type polysaccharides has been shown in A.centralis and C.filiformis, and evidence of a laminarin like polymer has been obtained in the investigations on C.rupestris and C.filiformis.

So far the green seaweeds appear from the chemical studies to fall into several main groups; those such as Cladophora and Chaetomorpha (Elizabeth Percival, unpublished work) species which synthesise arabinose containing polysaccharides and others such as Ulva lactuca and Acrosiphonia species which are devoid of arabinose. Whereas the arabinose containing green seaweeds, Cladophora and Chaetomorpha, appear to lack uronic acid residues, Ulva and Acrosiphonia have a relatively high uronic acid content, and it may be that the uronic acid containing polysaccharides fulfil the same function in these green algae as alginic acid does in the brown algae. Species of Caulerpa, on the other hand, contain very little arabinose and apparently very little, if any, uronic acid. The skeleton structure in this latter weed appears to be the 1,3-linked xylan which may comprise as much as 10% of the dry weight of the weed.

Carrageenin.

Earlier work by the author (Ph.D. thesis, Edin. 1941) on carrageenin, the polysaccharide of the red alga, Chondrus crispus, had established that the major portion of the polysaccharide molecule consisted of 1,3'-linked galactose units. This, however, only accounted for two thirds of the organic material present and 20% of the hydrolysate gave typical ketose colour reactions. Although 3,6-anhydro-L-galactose, the sugar unit present in agar, also gives these colour reactions, all attempts to isolate this derivative from the hydrolysate of carrageenin were unsuccessful. In 1953 Araki and Hirase (Bull.chem.Soc.Japan, 1953, 26, 463) isolated crystalline diethyl mercaptal 3,6-anhydro-L-galactose from the mercaptolysis products of agar. Similar experiments applied to carrageenin (12) have led to the isolation of the diethyl mercaptal of 3,6-anhydro-D-galactose indicating for the first time that the anhydride of D-galactose formed an integral part of the carrageenin molecule.

Laminarin.

Use was made of Barry degradation to determine whether the β-1,6'-glucosidic linkages in laminarin (Peat, Whelan and Lawley, J., 1958, 729), Anderson, Hirst, Manners and Ross, ibid. p.3233) occur as anomalous linkages in the β-1,3'-

linked chains or whether they serve as branch points (23). Any 1,6'-linked units in the chain would be cleaved by periodate and removed on subsequent treatment with phenylhydrazine and as a result the chains would be broken wherever these units occur. After three oxidations and two degradations the residual oxopolysaccharide still retained the characteristic property of insoluble laminarin, of slow precipitation from aqueous solution, and was sufficiently large to be retained inside a dialysis sac. Unless the 1,6'-links occur exclusively near the ends of the chains these results can only be explained by a branched structure for the laminarin molecule.

Residual polysaccharides isolated after Barry degradation always retain nitrogen-containing residues, and the proportion of this nitrogen is reduced on subsequent oxidation. While part of this nitrogen is probably present in the modified grouping at the reducing ends of the chains it has been shown (Finan and O'Colla, Chem. and Ind., 1958, 493) that when 1,6'-linked side-chains are joined to a central backbone, a bis-phenylhydrazone fragment remains after degradation attached to the degraded material. To obtain information on the nature of the nitrogen-containing residues and quantitative data for the amount of periodate

reduced by these groups on repeated oxidation, the action of periodate on the phenylhydrazones of a number of monosaccharides was measured (30). Comparison of the ultraviolet spectra of the hydrazones with those of the degraded polysaccharides showed that the two could not be related.

Alginic Acid.

A recent publication by Fischer and Dörfel (Z.physiol. Chem., 1955, 302, 186) claiming the isolation of L-gulurone (m.p. 142-3°) in addition to D-mannurone (m.p. 192°) from the hydrolysate of alginic acid, the main polysaccharide of the brown seaweeds, led to a reinvestigation by the candidate of the constitution of this polyuronide (24). Preliminary chromatographic experiments on the hydrolysate of various samples of alginic acid revealed the presence of two spots, one running at the same speed as authentic mannurone (m.p. 191°) and a second faster spot with the R_G recorded by Fischer and Dörfel for the lower melting lactone.

Reduction of the ester glycoside of alginic acid, followed by hydrolysis, gave a syrup containing mannose and also material which moved at the same speed as gulose on ionophoresis in borate buffer. In order to ensure that the gulose was not an artefact of acid hydrolysis and to obtain

information on the way in which the guluronic acid units were linked to the rest of the molecule, alginic acid was oxidised by periodate, and the resulting polyaldehyde converted to the polyacid by further oxidation with bromine. A polysaccharide containing both 1,4'-linked D-mannuronic acid and 1,4'-linked L-guluronic acid when subjected to this treatment should yield a polyacid which on hydrolysis would give a molecule of mesotartaric acid from each of the original 1,4'-linked D-mannuronic acid units and a molecule of L+-tartaric acid from the L-guluronic acid residues. Both meso- and L(+)-tartaric acid have indeed been isolated from a sample of alginic acid, isolated from Laminaria digitata, thus proving that 1,4'-linked L-guluronic acid residues are an integral part of the alginic acid molecule.

ANALYSIS OF POLYSACCHARIDES STRUCTURALLY RELATED TO THE ALGAL POLYSACCHARIDES

The amount of polysaccharide material available from the green seaweeds, the highest recorded yield being 12% of the dry weight, is often very small and this makes structural investigations difficult. The polysaccharides of the gum exudates present many of the same problems as the green algae polysaccharides. They contain a wide

variety of sugar units together with glucuronic and/or galacturonic acid residues. In undertaking structural investigations of the gum exudate of Brachychiton diversifolium (22) and of Albizia zygia it was hoped that, besides providing information on the structure of these two gums, valuable experience in the investigation of this type of material might be gained and that aldobiuronic acids and oligosaccharides would be isolated and characterised which would subsequently serve as reference compounds for similar fragments isolated in the seaweed polysaccharide work.

B.diversifolium gum exudate was shown to contain rhamnose (1 part), galactose (1 part) and glucuronic acid (2 parts). It resembles Sterculia setigera and Khaya grandifolia gums in its high uronic acid content, but differs in that the species of Sterculia gums examined contain galacturonic acid and in addition, in the case of Khaya, 4-O-methylglucuronic acid. The isolation and characterisation of 2-O-glucuronosyl-L-rhamnose from a partial acid hydrolysate of B.diversifolium gum established, for the first time, the presence of this residue in a gum molecule. Methylation studies confirmed the presence of this aldobiuronic acid unit and demonstrated that both galactose and

glucuronic acid units occur at the ends of the polysaccharide chains, that much of the galactose is present as 1,4'-linked units and that a considerable proportion of the rhamnose occurs at branch points in the molecule linked through C_1, C_2 and C_4.

Experiments on the gum exudate of Albizia zygia (D.W. Drummond and Elizabeth Percival, unpublished work) have proved that about 24% of the weight is made up of uronic acid residues. Galactose, arabinose, mannose and rhamnose in the ratio of 2.5:2:2:0.2, together with glucuronic acid and 4-O-methylglucuronic acid, have been separated from the gum hydrolysates and characterised as crystalline derivatives. Autohydrolysis removes a large proportion of the arabinose residues.

Oligosaccharides from the autohydrolysis, partial acid hydrolysis, and acetolysis experiments, are in course of separation and characterisation. The neutral sugar content of this gum is very similar to that of the water-soluble polysaccharide from Caulerpa filiformis and some of the partial hydrolysis fragments of the two polysaccharides appear to be identical.

APPLICATION OF MONOSACCHARIDE CHEMISTRY TO POLYSACCHARIDE PROBLEMS

Methyl Ethers of the Monosaccharides.

Early in these investigations it became evident that before methylation and periodate oxidation studies on algal polysaccharides could be pursued satisfactorily, many more partly methylated sugars and uronic acid reference compounds required to be synthesised, and their chromatographic behaviour established. For this reason the following syntheses were undertaken.

In order that authentic syntheses could be carried out, appropriate hydroxyl groups were blocked by suitable residues before methylation. Use was made of isopropylidene and toluene-p-sulphonyl blocking groups in the preparation of 3,4-di-O-methyl-L-fucose, 2,3-di-O-methyl-L-rhamnose (3) and 3,4- and 3,5-di-O-methyl-D-xylose (5). Three crystalline substances, whose constitutions had previously been established, were used as starting materials in the synthesis of three tri-O-methyl-, two di-O-methyl- and one mono-O-methyl ethers of fructose (8). These were 1:2, 4:5- and 2:3, 4:5-di-O-isopropylidene-D-fructose, and 2,3-O-isopropylidene-4,6-di-O-toluene-p-sulphonyl-D-fructose. In the course of this work some fifteen new crystalline derivatives

of fructose were synthesised.

Cleavage of the epoxide ring in methyl 2,3-anhydro-α-D-lyxofuranoside (13) and its 5-O-methyl-α- and β-derivatives (18) with methanolic ammonia resulted, after appropriate treatment, in the isolation of crystalline methyl 3-amino-3-deoxy-5-O-methyl- and -2,5-di-O-methyl-β-D-arabinoside.

Glycosidation followed by chromatographic separation led to the isolation of the four possible glycosides of L-fucose (21). Partial methylation of methyl β-L-fucofuranoside and of α-L-fucopyranoside followed by separation on cellulose columns resulted in the isolation and characterisation of eight of the eleven possible methyl ethers of fucose.

4-O-Methyl-D-mannuronic acid and 2,3-di-O-methyl-D-glucuronic acid were synthesised from mannose and glucose respectively (14). Methyl 2,3,4-tri-O-benzoyl-α-D-mannoside, synthesised for the first time, was utilized in the former of these two syntheses. In the second preparation, methyl 4,6-O-benzylidene-α-D-glucoside was the starting material. 2-O-Methyl and 3,4-di-O-methyl-D-galacturonic acid were synthesised from D-galactose (7) via 1,2:3,4-di-O-<u>iso</u>propylidene-D-galactose and -D-galacturonic

acid. These syntheses provided twelve new crystalline reference compounds of the uronic acids.

Investigation of the course of oxidation by periodate of different synthetic methyl ethers of the monosaccharides (9, 21, 29) yielded information which facilitated interpretation of the results of oxidation of the algal polysaccharides.

Toluene-p-sulphonyl and Epoxide Derivatives of Monosaccharides.

One of the major problems of algal polysaccharides is the determination of the position and function of the sulphate groups in the polysaccharide molecule. Furthermore the isolation of 2,5-di-O-methyl-3,6-anhydro-L-galactonic acid from the hydrolysis products of methylated agar (Jones and Peat, J., 1942, 225; E.G.V. Percival and Thomson, ibid., 750) showed that 3,6-anhydro-L-galactose units linked through position 4 were an integral part of the agar molecule. It is considered that these residues may be formed on the removal of the sulphate groups from the polysaccharide molecule during extraction or by enzymic action. It had already been demonstrated by Duff and E.G.V. Percival (J., 1941, 830) that alkaline hydrolysis of sulphate groups attached to hexoses can occur with formation of the 3,6-anhydro derivative of the parent sugar. On the other hand,

experiments (Haworth, Hirst, and Panizzon, J., 1934, 154; Oldham and Robertson, J., 1935, 685; Peat and Wiggins, J., 1938, 1088) on the alkaline hydrolysis of toluene p-sulphonyl (tosyl) groups from methyl glucosides had shown that the removal of a tosyl residue is often accompanied by a Walden inversion and this is invariably accompanied by anhydro ring formation provided that there is a free hydroxyl group in the trans position on the carbon atom adjacent to that carrying the tosyl group.

In order that a better understanding of the behaviour of the sulphated units in algal polysaccharides could be obtained and the presence of any anhydro sugars in the molecule be recognised and characterised, studies on monosaccharide sulphates and the anhydrides formed on their alkaline hydrolysis were undertaken. In view of the very similar behaviour of sugar sulphates and the toluene sulphonic esters of sugars, which are more easily prepared in the crystalline state, a variety of tosyl esters of the sugars most commonly found in the algal polysaccharides were synthesised, and their behaviour with alkali examined.

Alkaline hydrolysis of 3-O-toluene-p-sulphonyl-2,4,6-tri-O-methylgalactose resulted in the removal of the tosyl group without inversion (1). The intermediate formation of a 1,3-anhydro ring was postulated followed by the preferential

cleavage of the C_1-O link of the anhydro ring and the formation of 2,4,6-tri-O-methylgalactose. Treatment of methyl 4-O-toluene-p-sulphonyl-L-rhamnopyranoside (10), 2-O-toluene-p-sulphonyl-L-fucopyranoside (10) and -D-xylo-furanoside (6) with alkali resulted in the isolation of crystalline methyl 3,4-anhydro-, methyl 2,3-anhydro-6-deoxy-L-talopyranoside and methyl 2,3-anhydrolyxofuranoside respectively. In each of these three sugars alkaline fission of the tosyl grouping had resulted in inversion on the carbon atom which carried that grouping.

A study of the cleavage of the epoxide rings with sodium methoxide revealed (6, 10) that both C-O bonds were broken in the 3,4-anhydro-6-deoxy-L-talose and 2,3-anhydro-D-lyxo-furanose derivatives, inversion occurred at the point of entry of the methoxyl residues and two new sugar derivatives were isolated from each anhydro-sugar. In contrast, preferential fission of the C_3-O bond occurred in 2,3-anhydro-6-deoxytalose. Blockage of the free hydroxyl group in the anhydro-sugars with a methyl residue influenced the point of cleavage of the pyranoside sugar epoxides, but the results could not be explained on conformational grounds.

As a result of these studies it may be deduced that alkaline hydrolysis of sulphate groups in polysaccharides

only occurs when the sulphate group is adjacent to a carbon atom which carries a free hydroxyl group trans to the ester grouping, the removal of the sulphate group resulting in the formation of an epoxy- or 3,6-anhydro- derivative of a new or the same sugar. Since in the sulphated polysaccharides so far examined the sulphate groups have proved stable to alkali, they cannot be situated on hydroxyl groups in which the above conditions hold. In the water-soluble polysaccharides from C.rupestris (28) it can therefore be predicted that end group galactose units with sulphate groups on C_3 are absent, and that any sulphate groups attached to galactose must be on C_4 of the 1,3'-linked galactose chains. Since few of the galactose units in this polysaccharide have C_4 blocked it follows from the relatively high sulphate content that arabinose units carry sulphate groups on C_4, as sulphate groups attached either to C_3 in 1,4'-linked arabinose chains or C_2/C_3 of end group arabinose units would be labile to alkali. This is the first evidence that sugar units other than galactose in algal polysaccharides are sulphated. Similar considerations applied to the sulphated polysaccharides from A.centralis locates sulphate groups on C_3 or C_4 of the rhamnose residues, and on C_4 of the galactose units in the polysaccharide from C.filiformis.

Sugar Phosphates.

The presence of phosphate in starch and the importance of sugar phosphates in metabolic reactions in land plants suggested that sulphate groups might fulfil similar functions in the algae. In an attempt to correlate the behaviour of sugar phosphates with the carbohydrate sulphates, the alkaline hydrolysis of methyl α-glucopyranoside 6-phosphate, methyl glucofuranoside 3-phosphate and iso-propylidene glucofuranose 3- and 6-phosphates was studied (2). In these experiments, although the phosphate esters were hydrolysed with greater difficulty than the sulphates, no evidence for the formation of anhydro-rings of any kind or of Walden inversion could be found.

The importance of glucosamine 6-phosphate in a wide variety of biosynthetic reactions and its possible occurrence in marine algal metabolism led the candidate to undertake the first authentic chemical synthesis of this material (11, 17). In the course of this work eight new crystalline reference compounds of glucosamine were prepared and the final product was completely characterised; its behaviour to periodate and to the Elson Morgan reaction was also examined.

The above researches illustrate how a study of mono-saccharides and their derivatives can be applied in the

structural investigations on algal polysaccharides. In particular, the methyl ethers have provided essential reference compounds in the analysis of the algal polymers, and the behaviour of the synthetic methylated sugars to periodate oxidation has served as models for similar structures in the polysaccharides. Finally, the studies on the alkaline hydrolysis of the toluene-p-sulphonates has enabled prediction of the location of the sulphate groups to be made in the water-soluble polysaccharides of Cladophora rupestris, Acrosiphonia centralis and Caulerpa filiformis.

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299. *The Hydrolysis of* 3-p-*Toluenesulphonyl Derivatives of Galactose.*

By E. E. Percival and E. G. V. Percival.

The preparation and alkaline hydrolysis of 3-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl galactose is described. A trimethyl methylhexoside was obtained on hydrolysis from which on methylation and suitable treatment a good yield of tetramethyl galactopyranose anilide was obtained and no gulose derivatives were detected. It is concluded therefore that Walden inversion does not take place in this case on removal of the *p*-toluenesulphonyl residue. The resistance to hydrolysis of 3-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl α-methylgalactoside with acid and alkali is discussed.

Oldham and Robertson (J., 1935, 685) and Peat and Wiggins (this vol., p. 1088) point out that in the sugar series the removal of a *p*-toluenesulphonyl residue is not necessarily accompanied by a Walden inversion, but that when inversion is observed it is invariably accompanied by anhydro-ring formation. Similar conclusions are drawn by Irvine and Robertson (*Rec. Trav. chim.*, 1938, **57**, 575), who also point out that no Walden inversion has ever been authenticated involving ring formation other than of the ethylene oxide type, although Hess and Neumann (*Ber.*, 1935, **68**, 1360) claimed to have isolated a derivative of idose by the alkaline hydrolysis of 4-*p*-toluenesulphonyl 2 : 3 : 6-trimethyl glucose, but this claim is disputed by Freudenberg and Braun (*Ber.*, 1935, **68**, 1988).

An opportunity to test the possibilities of propylene oxide ring formation arose by the isolation of 3-p-*toluenesulphonyl* 2 : 4 : 6-*trimethyl galactose* from the corresponding *methylgalactoside* by the method of Hess and Neumann (*loc. cit.*) *via* the 1-*chloro*-3-p-*toluenesulphonyl* 2 : 4 : 6-*trimethyl galactose*. As isolated, this substance was chiefly but not exclusively the β-form, but it has not yet been possible, as with the original galactoside (Percival and Somerville, J., 1937, 1615), to separate a pure stereochemical form by fractional crystallisation. The subsequent arguments, however, are not affected by the presence of some of the α-variety.

A crystalline anhydro-compound could not be obtained by treatment of 3-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl galactose with sodium methoxide under various conditions, but with 5% sodium methoxide in methyl alcohol at 70° for 20 hours, and after suitable treatment, a syrup corresponding in properties to a trimethyl methylhexoside (A) was obtained in 50% yield. Complete methylation, hydrolysis, and anilide formation resulted in the isolation of tetramethyl galactopyranose anilide in good yield, and no other sugar could be detected. It must be admitted that, as no crystalline reference compounds of the other possibility, tetramethyl gulose, are yet available, a small quantity of this substance may have escaped notice, but (A) was undoubtedly mainly a mixture of trimethyl α- and β-methylgalactosides. The formation of this substance could be explained by the splitting off without inversion of the *p*-toluenesulphonyl residue, together with the "active" hydrogen atom on C_1 and the simultaneous entry of a methyl group in this position. Probably a more satisfactory explanation is that the *p*-toluenesulphonyl residue is eliminated from the 3-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl β-galactose with its two potential *cis*-hydroxyl groups, without Walden inversion, to yield the 1 : 3-anhydride, and this subsequently breaks down in one direction only, *viz.*, at the link to C_1. Otherwise, tetramethyl gulose or tetramethyl galactose (if two inversions had taken place on C_3) and not trimethyl methylgalactoside would have resulted.

It might have been expected that the breaking of the hypothetical β-1 : 3-anhydro-ring would yield an α-galactoside, since rupture of an ethylene oxide ring with sodium methoxide involves a Walden inversion at the point of entry of the new methyl group (Robertson and Griffith, J., 1935, 1193; Peat and Wiggins, *loc. cit.*). In this case the inversion is only partial, presumably owing to the extra mobility of the groups on C_1 as distinct from those on other carbon atoms. A partial inversion on C_3 when 2-acetyl 6-trityl 3 : 4-anhydro-α-methylgalactoside is treated with sodium methoxide is recorded by Oldham and Robertson (*loc. cit.*) although Peat and Wiggins (*loc. cit.*) obtained

complete inversion with 2 : 6-dimethyl 3 : 4-anhydro-β-methylalloside, and it may also be recalled that Levene and Raymond (*J. Biol. Chem.*, 1933, **102**, 317) converted 1 : 2-monoacetone 3 : 5-anhydro-xylofuranose into 1 : 2-monoacetone 5-methyl xylofuranose by treatment with sodium methoxide. The present results show therefore that Walden inversion does not occur on the removal of a *p*-toluenesulphonyl residue from C_3 although a *cis*-hydroxyl group is available on C_1, and have a parallel in the observation of Peat and Wiggins (*loc. cit.*) that 3-*p*-toluenesulphonyl glucofuranose monoacetone may also be hydrolysed without Walden inversion.

Finally, we record that 3-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl methylgalactoside was remarkably resistant to hydrolysis, although after 100 hours' treatment with sodium methoxide at 70°, crystalline 2 : 4 : 6-trimethyl methylgalactoside was isolated, showing, as expected, the absence of Walden inversion. Attempts to hydrolyse the glycosidic methoxyl residue with 7% aqueous-alcoholic sulphuric acid at 100° for as long as 50 hours were futile, much of the starting material being recovered unchanged. Although the galactoside in question contained some of the β-form, it was largely the α-variety, and the results are in contrast to the behaviour of the corresponding β-galactoside, which is readily hydrolysed by sodium methoxide (Bell and Williamson, this vol., p. 1196). From an inspection of models it would seem likely that the *p*-toluenesulphonyl residue in the α-form can approach so closely to the glycosidic hydrogen atom (*cis*) as to affect its properties. This could occur either by virtue of the purely mechanical shielding effect of the large aromatic residue or, more probably, by the formation of a bond, due to the existence of two forms in resonance, between the glycosidic hydrogen atom and the strongly electronegative oxygen atoms of the *p*-toluenesulphonyl residue. In the β-form the methoxyl residue is *cis*- and the hydrogen atom is *trans*- to the *p*-toluenesulphonyl residue on C_3, so the effect is not observed in this case.

Experimental.

3-p-*Toluenesulphonyl* 2 : 4 : 6-*Trimethyl Methylgalactoside*.—2 : 4 : 6-Trimethyl methylgalactoside (2 g.), m. p. 40—50°, $[\alpha]_D^{20°}$ + 108° in water (*c*, 0·6), was dissolved in pyridine (2·5 c.c.) and treated with *p*-toluenesulphonyl chloride (2·4 g.) at 0°. After 24 hours, water was added, and the mixture extracted with benzene, the extract being washed with dilute hydrochloric acid, sodium bicarbonate solution, and water, and dried over sodium sulphate. After removal of solvent under diminished pressure, a syrup was obtained which crystallised spontaneously (2·75 g.) and on recrystallisation from light petroleum (b. p. 60—80°) gave shining needles, m. p. 119—120°, $[\alpha]_D$ + 84° in chloroform (*c*, 0·5) (Found: C, 52·1; H, 7·0; OMe, 31·0. $C_{17}H_{26}O_8S$ requires C, 52·3; H, 6·7; OMe, 31·8%).

1-*Chloro*-3-p-*toluenesulphonyl* 2 : 4 : 6-*Trimethyl Galactose*.—The foregoing crystalline 3-p-*toluenesulphonyl* 2 : 4 : 6-*trimethyl methylgalactoside* (1 g.) was dissolved in acetic anhydride (6 c.c.), and the solution saturated with dry hydrogen chloride at − 18°. The containing tube was then sealed, and kept at room temperature for 3 days. On pouring the mixture on ice, a solid was obtained which was dissolved in benzene, washed with sodium bicarbonate solution, dried with sodium sulphate, and the benzene removed (diminished pressure) to yield a colourless syrup which crystallised spontaneously. The product (1·2 g.), after one recrystallisation from light petroleum (b. p. 60—80°), had m. p. 108°, $[\alpha]_D^{20°}$ + 136° in acetone (*c*, 0·5) (Found: C, 49·0; H, 6·1; OMe, 23·9; Cl, 9·7. $C_{16}H_{23}O_7ClS$ requires C, 48·7; H, 5·9; OMe, 23·6; Cl, 9·0%).

3-p-*Toluenesulphonyl* 2 : 4 : 6-*Trimethyl Galactose*.—The above *chloro*-compound (1 g.), dissolved in 90% aqueous acetone (10 c.c.), was shaken for 50 hours with silver carbonate (5 g.). After filtration and evaporation at 40°/20 mm., a crystalline mass was obtained which was recrystallised from methyl alcohol, yielding 3-p-*toluenesulphonyl* 2 : 4 : 6-*trimethyl galactose* (0·7 g.), m. p. 138°, $[\alpha]_D^{20°}$ in chloroform (*c*, 0·53) + 30° ⟶ + 96° in 96 hours (Found: C, 51·3; H, 6·6; OMe, 24·5. $C_{16}H_{24}O_8S$ requires C, 51·1; H, 6·4; OMe, 24·7%).

Alkaline hydrolysis. In a typical experiment, 3-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl galactose (2·5 g.) in methyl alcohol (60 c.c.) containing sodium (1·5 g.) was heated at 70° for 20 hours. After the addition of water (5 c.c.), carbon dioxide was passed through the mixture for 1 hour, the precipitate filtered off, solvent removed under diminished pressure, and the product thoroughly extracted with boiling chloroform (1 l.). After removal of solvent, the

syrup (1·6 g.) was transferred to a small flask by means of ether, and finally distilled: (1) 0·8 g., b. p. 95—105° (bath temp.)/0·05 mm.; (2) 0·2 g., b. p. 105—150°; (3) residue, 0·5 g. Fraction (1) was non-reducing until warmed with dilute acid, and had $n_D^{20°}$ 1·4553, $[\alpha]_D^{20°}$ + 50° in water (*c*, 0·5) (Found: C, 50·4; H, 8·7; OMe, 52·3. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%). Fraction (2) had $n_D^{20°}$ 1·4650 and was faintly reducing; the quantity was insufficient for detailed examination. Hydrolyses carried out as above for periods of 2, 5, and 8 hours afforded (*a*) 8, (*b*) 38, and (*c*) 42% of the theoretical yields of the tetramethyl compound respectively; (*a*) was slightly reducing and may have contained the free anhydro-compound, but the poor yield precluded further investigation; the specific rotations of (*b*) and (*c*) were lower ($[\alpha]_D$ + 30°) than for the case given above, a fact which was thought to point to the presence of gulose derivatives, but tetramethyl galactose anilide was isolated from (*c*) as well as from the product of 20 hours' treatment as described below.

Methylation, and the Isolation of Tetramethyl Galactopyranose Anilide.—The substance from the 20 hours' treatment, having failed to crystallise, was subjected to two methylations by the Purdie method; the product (yield, quantitative) distilled completely at 95° (bath temp.)/0·05 mm. and showed $n_D^{19°}$ 1·4473, $[\alpha]_D^{20°}$ + 75° in water (*c*, 0·9) (Found: OMe, 60·6. Calc. for $C_{11}H_{22}O_6$: OMe, 62·0%).

After hydrolysis for 2 hours with 6% hydrochloric acid and neutralisation with silver carbonate, a portion of the reducing syrup so obtained (0·5 g.) was heated with aniline (0·4 g.) in alcohol (4 c.c.) for 4 hours at 100°. The crystalline anilide which separated on cooling was filtered off (0·4 g., m. p. 194°) and recrystallised once; m. p. 197°, unchanged on admixture with authentic tetramethyl galactopyranose anilide (Found: OMe, 40·0; N, 4·5. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·8; N, 4·5%). The free sugar {0·1 g., $[\alpha]_D^{20°}$ + 80° in water (*c*, 0·3)}, isolated from the mother-liquors by addition of hydrochloric acid and suitable treatment, was examined for the presence of gulose derivatives but none was found.

Treatment of 3-p-*Toluenesulphonyl* 2 : 4 : 6-*Trimethyl Methylgalactoside with Sodium Methoxide.*—Hydrolysis was carried out at 70° in a 5% solution of sodium methoxide, the product being isolated by dilution of the reaction mixture with 3 vols. of water, followed by extraction with chloroform. Hydrolysis for 12 or for 24 hours was unsuccessful, the original material being recovered unchanged. Treatment for 100 hours, however, gave rise to the original trimethyl methylgalactoside, m. p. 45—50°, $[\alpha]_D^{20°}$ + 106° in water. Yield 40% of the theoretical.

Attempted Acid Hydrolysis.—A typical experiment is described. 3-*p*-Toluenesulphonyl 2 : 4 : 6-trimethyl methylgalactoside (0·4 g.) was dissolved in alcohol (5 c.c.), water (10 c.c.) and concentrated sulphuric acid (0·6 c.c.) were added, and the mixture was heated under reflux for 24 hours at 100°. On cooling, crystallisation ensued, and unchanged material (0·25 g.), m. p. 119°, was filtered off. After neutralisation of the mother-liquors with barium carbonate and evaporation to dryness, a further small quantity (0·05 g.) of unchanged material separated, but no reducing product was obtained. Similar results were obtained when the duration of the hydrolysis was 2, 3, 9, and 50 hours. Even on continuing the treatment for 95 hours at 90° it was still possible to isolate some crystalline starting material, although decomposition had undoubtedly occurred, since the final syrup was brown and reducing. A control experiment on 2 : 4 : 6-trimethyl methylgalactoside with 7% aqueous-alcoholic sulphuric acid under the same conditions showed that in this case hydrolysis was complete in less than 3 hours.

Attempts to prepare 1 : 3-di-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl galactose directly from 2 : 4 : 6-trimethyl α-*d*-galactose were unsuccessful, only the 3-*p*-toluenesulphonyl derivative being isolated in poor yield.

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232. *Carbohydrate Phosphoric Esters. Part I. The Alkaline Hydrolysis of α-Methylglucopyranoside-6-phosphate, Methylglucofuranoside-3-phosphates and iso-Propylidene Glucofuranose-3- and -6-phosphates.*

By E. E. Percival and E. G. V. Percival.

The alkaline hydrolysis of barium α-methylglucopyranoside-6-phosphate, barium methylglucofuranoside-3-phosphates and barium *iso*propylidene glucose-3- and -6-phosphates has been studied and in no case was any evidence found of Walden inversion or anhydride formation.

Although Robinson (*Nature*, 1927, **120**, 44, 656) made the attractive suggestion that the hydrolysis of phosphoric esters with accompanying Walden inversion might account for the production in nature of *d*-galactose

and *d*-ribose from glucose-4-phosphate and xylose-3-phosphate respectively, no evidence for Walden inversio in the hydrolysis of sugar phosphates has been recorded (Gardner, *J. Org. Chem.*, 1943, **8**, 111; Raymon "Organic Chemistry," Gilman, New York, 1938, 1480).

In all cases where Walden inversions occur with sugar derivatives with the exception of compounds su stituted on the potential reducing groups, the intermediate formation of a ring system such as the ethyle oxide ring is necessary, as in the hydrolysis of appropriately substituted toluene-*p*-sulphonates (Peat, *Annu Rep.*, 1939, 258). From experiments on the alkaline hydrolysis of carbohydrate sulphates it would appear th Walden inversions do not take place and ethylene oxide rings are not formed, although **3** : 6-anhydrides we isolated by the alkaline hydrolysis of the methylglycopyranosides of barium glucose-, galactose-, and mannos 6-sulphates, methylglucofuranoside-3- and -6-sulphates and **1** : 2-monoacetone glucofuranose-6-sulphate (D and Percival, *J.*, 1941, 830; Percival, this vol., p. 119). In the experiments here described, however, no eviden for the formation of anhydro rings of any kind or of Walden inversions could be found when phosphoric este of certain glucose derivatives were hydrolysed with alkali. Furthermore the phosphate residues were r moved with greater difficulty than for the corresponding sulphates. The results may be summarised thus (1) barium α-methylglucopyranoside-6-phosphate gave α-methylglucopyranoside (47%) after hydrolysis f 72 hours with N-barium hydroxide solution at 100°; (2) barium methylglucofuranoside-3-phosphates (αβ-mi ture) gave methylglucofuranosides (52%); (3) barium **1** : 2-monoacetone glucofuranose-3-phosphate ga **1** : 2-monoacetone glucofuranose (11%); (4) barium **1** : 2-monoacetone glucofuranose-6-phosphate the sa product (22%). Substantial amounts of the unchanged starting materials were recovered from experimen (3) and (4). The product obtained from (2), when hydrolysed with dilute acid, gave crystalline α-*d*-gluco together with a syrup, but from this latter on osazone formation glucosazone was the only substance isolate A syrup also accompanied the monoacetone glucofuranose obtained in (4); in this case also, glucosazone was t only product identified after hydrolysis and osazone formation. No **3** : 6-anhydroglucosazone was fou although the detection of this substance has been simplified by the discovery that **3** : 6-anhydroglucosazo acetate is more readily crystallisable than the parent osazone.

It should be pointed out that Seebeck, Meyer, and Reichstein (*Helv. Chim. Acta*, 1944, **27**, 1142) have thro doubt on the conclusions of Ohle and von Vargha (*Ber.*, 1929, **62**, 2435) that *l*-idose can be obtained fr **1** : 2-*iso*propylidene-5 : 6-anhydroglucose, since this latter substance is readily converted into the correspondi **3** : 6-anhydride. If this is the case, the possibility that the alkaline hydrolysis of the methylglucofuranoside sulphates and the **1** : 2-*iso*propylidene glucofuranose-6-sulphate (Percival, *loc. cit.*) might take place *via* t **5** : 6-anhydride cannot be ignored although the failure to isolate *l*-sorbosazone was held to discount this pos bility. Owing to the fact that no **3** : 6-anhydroglucosazone was isolated in the present investigation th difficulty does not arise here.

In conclusion, reference may be made to the isolation by Levene and Raymond (*J. Biol. Chem.*, 1929, 8 619) of an anhydrohexosazone, in unspecified yield, from barium glucose-3-phosphate prepared by the a hydrolysis of diacetone glucose-3-phosphate; despite several attempts we have been unable to repeat this wo The reported properties of the anhydro-osazone in question, however, are not inconsistent with the fact th it might have been the anhydroglucosazone isolated by Diels and Meyer (*Annalen*, 1935, **519**, 157) on heati glucosazone in alcohol with a trace of sulphuric acid, and it may be noted that before treatment with phen hydrazine and acetic acid, Levene and Raymond removed barium from their salt with this reagent. Altho Diels and Meyer (*loc. cit.*) claimed their anhydroglucosazone to be identical with **3** : 6-anhydroglucosazone i now known (Percival, this vol., p. 783) that this is certainly not the case; it appears to us that the isolation of anhydrohexosazone by Levene and Raymond (*loc. cit.*) following the acid hydrolysis of a phosphoric ester m not in fact have been directly connected with the hydrolysis of such an ester but with some abnor condition prevailing during osazone formation.

Experimental.

Preparation of Barium α-Methylglucopyranoside-6-phosphate.—α-Methylglucopyranoside (m. p. 166°) (10 g.) was c verted into barium α-methylglucoside-6-phosphate according to the method of Fischer (*Ber.*, 1914, **47**, 3193). A continuous extraction with alcohol under reflux for 24 hours the product, dissolved in the minimum quantity of wa was filtered into alcohol to give a white precipitate which was separated and dried (11 g.). It had $[\alpha]_D^{16°}$ +95° (*c*, in water) (Found: OMe, 7·2; P, 7·6. Calc. for $C_7H_{13}O_9PBa$: OMe, 7·6; P, 7·6%).

Alkaline Hydrolysis.—Barium α-methylglucoside-6-phosphate (4·6 g.) was hydrolysed in water (150 c.c.) at with barium hydroxide (24 g.) for 72 hours. The alcohol soluble portion of the hydrolysate gave a colourless crystal solid (1·45 g., 47%); this was recrystallised from hot alcohol and the crystals (m. p. 166°) obtained were identifie α-methylglucoside (mixed m. p.) and had [α] +153° (*c*, 1·5, in water). The hydrolysis was repeated a number of t but all attempts to isolate any other product were unsuccessful.

Preparation of Barium **1** : 2-*Monoacetone Glucofuranose-6-phosphate and its Hydrolysis with Alkali.*—Monoace glucose (5 g.) in dry pyridine (18 c.c.) was converted into monoacetone glucose-6-phosphate according to the method for the preparation of diacetone glucose-3-phosphate. The product, after treatment with silver sulphate, was extra with hot ethyl acetate, dissolved in water, filtered and the water removed by distillation at 40°/15 mm. to yield a w solid (4·8 g.), $[\alpha]_D^{15°}$ +12° (*c*, 1·4 in water) (Found: Ba, 29·6; Me_2CO, 13·8. Calc. for $C_9H_{15}O_9PBa$: Ba, 31·5; Me 13·3%).

The above product (3·5 g.) was hydrolysed with barium hydroxide at 100° as before. The product was extra thoroughly with ethyl acetate and left a residue which was dissolved in water, filtered and evaporated to give a w solid (1·36 g.), $[\alpha]_D^{17°}$ +10° (*c*, 1·2 in water) [Found: Ba, 27·9; Me_2CO, 12·5%]. This was largely unchanged mate Evaporation of the ethyl acetate extracts gave a residue (0·55 g.) which, after extraction several times with cold dry et left a crystalline residue (0·35 g.), m. p. 162°, identified as **1** : 2-monoacetone glucofuranose; $[\alpha]_D^{18°}$ −11° (*c*, 1·5 in wa

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When the ether was removed from the ethereal extracts a colourless syrup (0·17 g.) remained. This, after standing for several weeks, partly crystallised. The crystals (0·04 g.) were separated on porous porcelain and had m. p. 159°, not depressed by monoacetone glucoses and the total yield was 0·39 g., 22% of the theoretical.

The syrup (0·12 g.) obtained from the porous porcelain was hydrolysed with 0·1N sulphuric acid (10 c.c.) at 45° for 47 hours. The product (0·1 g.), isolated in the usual way, had $[\alpha]_D^{15°}$ +44° (*c*, 1·0 in water) and was subjected to osazone formation to give four crops of osazone (0·043 g.) having m. p. 165—170°, depressed to 140° on admixture with *l*-sorbosazone (m. p. 165°). After several crystallisations from alcohol a small amount of crystalline product having m. p. 185—186° was obtained; m. p. depressed to 170° by admixture with 3 : 6-anhydroglucosazone (m. p. 185°). Acetylation with acetic anhydride and pyridine at room temperature failed to yield any crystallisable acetate, and, in particular, the easily crystallisable 3 : 6-anhydroglucosazone acetate. This work was repeated three times, but it was impossible to obtain identifiable products other than glucosazone and 1 : 2-monoacetone glucose.

Barium Diacetone Glucofuranose-3-phosphate.—Pure diacetone glucose (10 g.) in dry pyridine (37·5 c.c.) was treated with phosphorus oxychloride (5 c.c.) in pyridine (25 c.c.) according to the method of Josephson and Proffe (*Annalen*, 1930, **481**, 91; cf. Raymond and Levene, *J. Biol. Chem.*, 1929, **83**, 619). After the addition of saturated barium hydroxide solution until alkaline to phenolphthalein and removal of the excess pyridine by distillation at 35°/15 mm. the solid product was dissolved in water (100 c.c.) and a saturated solution of barium hydroxide added until faintly alkaline to thymolphthalein. The solution was decolorised with charcoal, filtered and the filtrate evaporated to give a white solid. Unchanged diacetone glucose was removed by repeated extraction with ligroin (6 litres) under reflux for 3 days. The residue, dissolved in water (75 c.c.), was treated with an excess of silver sulphate, the solution filtered, the excess of silver ion removed by hydrogen sulphide in the presence of barium carbonate, followed by aeration, treatment with barium hydroxide and carbon dioxide. The resulting solution, after filtration, was evaporated to dryness at 40°/15 mm. to yield a non-reducing white solid. This was purified by solution in acetone and precipitation with ligroin (b. p. 40—60°), followed by several extractions with the same solvent. The product (12·5 g.) had $[\alpha]_D^{18°}$ −3·6° (*c*, 1·0 in water) (Found : Ba, 26·0; P, 6·9; Me_2CO, 25·0. Calc. for $C_{12}H_{19}O_9PBa$: Ba, 28·9; P, 6·5; Me_2CO, 24·4%). The product contained therefore a small amount of the acid phosphate (cf. Josephson and Proffe, *loc. cit.*).

Barium 1 : 2-Monoacetone Glucofuranose-3-phosphate and its Hydrolysis with Alkali.—The above salt (12 g.) was dissolved in water (50 c.c.) and treated with 0·5N sulphuric acid (70 c.c.) for 165 minutes at room temperature. The solution, made alkaline to thymolphthalein with saturated barium hydroxide (30 c.c.), was treated with carbon dioxide, filtered, and the filtrate and washings on evaporation gave a yellow solid which was extracted with ethyl acetate under reflux for 14 hours to leave a pale yellow residue (9·8 g.), $[\alpha]_D^{15°}$ +8° (*c*, 1·7, in water) (Found : Ba, 30·8; Me_2CO, 13·1. Calc. for $C_9H_{15}O_9PBa$: Ba, 31·5; Me_2CO, 13·3%).

Barium 1 : 2-monoacetone glucose-3-phosphate (3 g.) was hydrolysed with barium hydroxide as before. After cooling and filtration, the excess of alkali was neutralised with carbon dioxide and the filtrate evaporated to dryness at 35°/15 mm. to give a white solid : this was extracted several times with ethyl acetate under reflux. The remaining white solid (1·94 g.) had $[\alpha]_D^{18°}$ +6° (*c*, 1·6, in water) (and was for the most part unchanged material) [Found : Ba, 29·6; Me_2CO, 13·2%]. The ethyl acetate extracts gave a white solid (0·164 g.) which was recrystallised from ether. It had m. p. 161°, and was identified as 1 : 2-monoacetone glucofuranose, $[\alpha]_D^{17°}$ −12° (*c*, 0·9, in water) (Found : Me_2CO, 25·5. Calc. for $C_9H_{16}O_6$: Me_2CO, 26·5%). The unchanged material was submitted to further hydrolyses with barium hydroxide but monoacetone glucose was the only compound isolated in each case.

This work was repeated three times with identical results.

Barium Glucose-3-phosphate.—Barium 1 : 2-monoacetone glucose-3-phosphate (4·55 g.) was dissolved in water (100 c.c.) and treated with N-sulphuric acid (25 c.c.) at 40° for 43 hours. The product was neutralised, filtered and evaporated to dryness at 35°/15 mm. to yield a white solid (3 g.), $[\alpha]_D^{15°}$ +29° (*c*, 1·0, in water) (cf. Josephson and Proffe, *loc. cit.*) (Found : Ba, 32·5. Calc. for $C_9H_{11}O_9PBa$: Ba, 34·7%).

Barium Methylglucofuranoside-3-phosphates and their Hydrolysis with Alkali.—Barium glucose-3-phosphate (3·5 g.) was shaken at 15° with methanolic hydrogen chloride (60 c.c., 1·3%) until the specific rotation had fallen to a constant value (120 hours). After neutralisation with barium carbonate and removal of the solvent the mixture of salts was dissolved in water and treated with excess silver sulphate and the product worked up in the usual way to yield a glass. This was extracted thoroughly with ethyl acetate under reflux to remove methylglucofuranosides and the *product* (2·5 g.) had $[\alpha]_D^{17°}$ +4° (*c*, 1·0, in water) (Found : Ba, 32·0; P, 7·0; OMe, 6·8. $C_7H_{13}O_9PBa$ requires Ba, 33·6; P, 7·6; OMe, 7·6%).

The above product (2·45 g.) was hydrolysed at 100° with barium hydroxide solution for 72 hours as before and the residue obtained, after neutralisation and evaporation, was extracted repeatedly with alcohol to give a syrupy glass (0·61 g.) (Found : OMe, 13·9. Calc. for $C_7H_{14}O_6$: OMe, 16·0%). The product (0·57 g.) was hydrolysed with 0·2N sulphuric acid (20 c.c.) at 100° for 7 hours. After neutralisation with barium carbonate, the solution was evaporated and extraction with alcohol yielded a reducing syrup (0·4 g.) which was purified by dissolution in water, filtration and evaporation, followed by drying with alcohol and benzene; the resulting product (0·36 g.) had $[\alpha]_D^{17°}$ +59·7° (*c*, 1·8 in water). On standing for some months this syrup partly crystallised. The crystals (0·1 g.), separated from the syrup by treatment with ethyl acetate, had m. p. 150°, not depressed by *d*-glucose (m. p. 150°). The remainder was subjected to osazone formation to give two crops of osazone (0·16 g.), m. p. 207° not depressed by an authentic specimen of glucosazone (m. p. 208°). Recrystallisation from alcohol and evaporation of the mother liquors gave a small quantity (0·03 g.) of a syrup which could not be crystallised. This syrup was acetylated with acetic anhydride and pyridine but the acetate so obtained could not be crystallised from aqueous alcohol in contrast with the easily crystallisable 3 : 6-anhydroglucosazone acetate.

Attempted Isolation of an Anhydrohexosazone from Barium Glucose-3-phosphate.—Barium glucose-3-phosphate (1·9 g.) was treated with the calculated quantity of sulphuric acid, filtered, and heated with sodium acetate (2 g.), phenylhydrazine (2 c.c.) and acetic acid (1·5 c.c.) at 100° for 45 minutes. A crystalline product (0·6 g.) was obtained, m. p. 118—120°, $[\alpha]_D^{17°}$ −62° (*c*, 0·2, in pyridine–alcohol) (Found : N, 16·3; P, 3·95%). The product could not be recrystallised and the analyical figures of three such preparations were too variable to permit the assignment of a formula. It appears highly probable, however, to be a phenylhydrazine salt of glucosephenylosazone-3-phosphate; cf. the corresponding derivative obtained from glucose-6-phosphate, m. p. 154—154·5° (Robison and King, *Biochem. J.*, 1931, 323). Attempts to isolate the anhydrohexosazone reported by Levene and Raymond (*loc. cit.*) were abortive.

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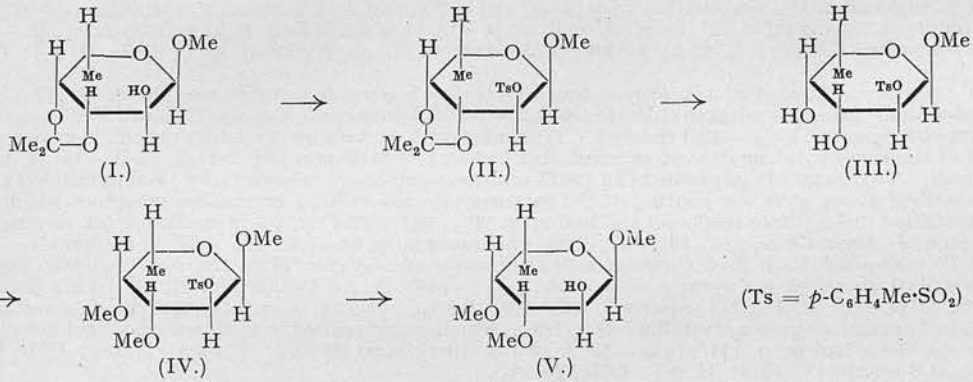
137. 3 : 4-*Dimethyl* L-*Fucose and* 2 : 3-*Dimethyl* L-*Rhamnose.*

By E. E. Percival and E. G. V. Percival.

From 2-*toluene*-p-*sulphonyl* 3 : 4-iso*propylidene* α-*methyl*-L-*fucoside* by mild hydrolysis, methylation, and reduction, 3 : 4-*dimethyl* α-*methyl*-L-*fucoside* and 3 : 4-*dimethyl* L-*fucose* have been prepared. 2 : 3-Dimethyl L-rhamnose has been prepared by a similar route.

The isolation of a dimethyl L-fucose from methylated fucoidin made it desirable to prepare synthetic dimethyl fucoses of known structure. The preparation and properties of the 3 : 4-dimethyl isomer are now recorded.

α-Methyl-L-fucoside was converted into the 3 : 4-*iso*propylidene derivative (I) (MacPhillamy and Elderfield, *J. Org. Chem.*, 1939, **4**, 150) from which 2-*toluene*-p-*sulphonyl* 3 : 4-iso*propylidene* α-*methyl*-L-*fucoside* (II) was prepared. Methanolysis removed the acetone residue (to give III), and methylation then gave 2-*toluene*-p-*sulphonyl* 3 : 4-*dimethyl* α-*methyl*-L-*fucoside* (IV). Reductive fission of the toluene-*p*-sulphonyl residue gave 3 : 4-*dimethyl* α-*methyl*-L-*fucoside* (V) from which by hydrolysis 3 : 4-*dimethyl* L-*fucose* was obtained as the monohydrate, m. p. 75°, $[\alpha]_D$ −118°.



By a similar process 2 : 3-*iso*propylidene α-methyl-L-rhamnoside was converted into 4-*toluene*-p-*sulphonyl* 2 : 3-*dimethyl* α-*methyl*-L-*rhamnoside* from which, by reductive hydrolysis followed by the removal of the glycosidic methyl group, 2 : 3-dimethyl L-rhamnose was prepared as a syrup, $[\alpha]_D$ +40°. This compound prepared by another route (Schmidt, Plankenhorn, and Kübler, *Ber.*, 1942, **75**, 579) is reported to have $[\alpha]_D$ +47·6°. Crystalline 2 : 3-*dimethyl* L-*rhamnose anilide* has been prepared.

By the paper chromatogram the R_G values (Hirst, Hough, and Jones, *J.*, 1949, 928) of both dimethyl 6-deoxyhexoses have been determined and compared with those of 2-methyl fucose and 4-methyl rhamnose, prepared by the methylation and hydrolysis of the corresponding *iso*propylidene α-methylglycosides. Certain differences between the results obtained and those

Sugar.	R_G.	Sugar.	R_G.
4-Methyl L-rhamnose	0·64; 0·65 (0·57)	2-Methyl L-fucose	0·56 (0·51)
2 : 3-Dimethyl L-rhamnose ...	0·82; 0·83	3 : 4-Dimethyl L-fucose	0·67; 0·68

previously published (Hirst, Hough, and Jones, *loc. cit.*) (in parentheses) emphasise the point made in that publication that such figures are useful as an indication of the methylated sugar concerned, but that direct comparisons on the same chromatogram are essential before identity (in the chromatographic sense) can be established, because of the difficulty of the rigid standardisation of all the variables between different laboratories.

Experimental.

3 : 4-iso*Propylidene* α-*Methyl*-L-*fucoside*.—α-Methyl-L-fucoside (1 g.) in acetone (50 c.c.) containing two drops of acetaldehyde was shaken with anhydrous copper sulphate (11 g.) for 120 hours. One drop of concentrated sulphuric acid was then added, and shaking continued for a further 14 hours. After

filtration and neutralisation with potassium carbonate, the solvent was evaporated; the product (0·9 g.), distilled at 95°/0·01 mm., had n_D^{15} 1·4621, $[\alpha]_D^{15}$ −160° (*c*, 1·0 in water). On storage in the refrigerator the product solidified.

2-*Toluene*-p-*sulphonyl* 3 : 4-iso*Propylidene* α-*Methyl*-L-*fucoside*.—The above derivative (0·51 g.) in pyridine (5 c.c.) was treated with powdered toluene-*p*-sulphonyl chloride (1 g.) and kept for 2 days at 15°. When the mixture was poured on ice a white crystalline solid was obtained which was combined with the product obtained on extraction of the filtrate with chloroform, which was washed with dilute sulphuric acid, aqueous sodium hydrogen carbonate, and water, dried, and evaporated. On recrystallisation from methanol a crystalline *product* (0·37 g.), m. p. 182°, $[\alpha]_D^{15}$ −146° (*c*, 1·0 in $CHCl_3$), was obtained (Found: C, 54·7; H, 6·5; OMe, 8·5. $C_{17}H_{24}O_7S$ requires C, 54·8; H, 6·45; OMe, 8·3%).

2-*Toluene*-p-*sulphonyl* 3 : 4-*Dimethyl* α-*Methyl*-L-*fucoside*.—Crystalline 2-toluene-*p*-sulphonyl 3 : 4-*iso*propylidene α-methylfucoside (0·5 g.) was treated with methanolic hydrogen chloride (10 c.c.; 1%) at 70° for 1 hour. After neutralisation with silver carbonate and extraction of the silver residues with ethanol, 2-*toluene*-p-*sulphonyl* α-*methyl*-L-*fucoside* (0·36 g.), m. p. 158°, $[\alpha]_D^{15}$ −85° (*c*, 1·0 in chloroform), was obtained. This substance was then methylated 4 times with methyl iodide and silver oxide, to give a crystalline *product* (0·4 g.) which, recrystallised from chloroform–light petroleum (b. p. 60—80°), had m. p. 103°, $[\alpha]_D^{15}$ −84° (*c*, 0·7 in chloroform) (Found: C, 53·6; H, 6·8; OMe, 16·6; S, 8·2. $C_{16}H_{24}O_7S$ requires C, 53·3; H, 6·7; OMe, 17·2; S, 8·9%).

3 : 4-*Dimethyl* α-*Methyl*-L-*fucoside*.—The above compound (0·5 g.) in methanol (11 c.c.) and water (5 c.c.) was treated with sodium amalgam (10 g.; 4%) with stirring at 35° during 17 hours. After filtration and extraction with chloroform, the extracts were treated with carbon dioxide for 15 minutes, dried (Na_2SO_4), and freed from organic solvents under diminished pressure. The aqueous residue was neutralised with carbon dioxide, evaporated to dryness, and extracted with ether. From these operations a crystalline *fucoside* was obtained (0·2 g.) which was recrystallised from boiling light petroleum (b. p. 40—60°), to give crystals, m. p. 100°, $[\alpha]_D^{20}$ −213° (*c*, 1·3 in water) (Found: C, 52·1; H, 8·5; OMe, 44·6. $C_9H_{18}O_5$ requires C, 52·4; H, 8·6; OMe, 45·1%).

3 : 4-*Dimethyl* L-*Fucose*.—3 : 4-Dimethyl α-methyl-L-fucoside (0·13 g.) was hydrolysed at 100° with sulphuric acid (15 c.c.; 4%) for 3 hours, whereafter the rotation was constant. After neutralisation with barium carbonate, evaporation to dryness, and extraction with acetone, a crystalline *ether* (0·1 g.) was obtained, having m. p. 82° (from ethanol), m. p. 76° (from chloroform–light petroleum), $[\alpha]_D^{15}$ −118° (*c*, 1·1 in water) (Found: C, 45·9; H, 8·9; OMe, 27·9. $C_8H_{16}O_5,H_2O$ requires C, 45·6; H, 8·6; OMe, 29·5%).

4-*Toluene*-p-*sulphonyl* 2 : 3-iso*Propylidene* α-*Methyl*-L-*rhamnoside*.—Anhydrous rhamnose (12 g.) was boiled with methanolic hydrogen chloride (150 c.c.; 0·25%) until non-reducing (40 hours) and the syrupy α-methylrhamnoside ($[\alpha]_D$ −48°) isolated. Treatment with acetone as previously described gave a good yield of the *iso*propylidene derivative which distilled at 110°/0·05 mm., n_D^{11} 1·4563, $[\alpha]_D^{12}$ −14° (*c*, 1·1 in acetone). Treatment of this product (3 g.) with toluene-*p*-sulphonyl chloride (5·5 g.) and pyridine (14 c.c.) as described above gave, on pouring of the mixture into ice-water, a crystalline substance which was recrystallised (2·7 g.) from methanol and had m. p. 60°, $[\alpha]_D^{15}$ +22·5° (*c*, 2·9 in methanol) (cf. Levene and Compton, *J. Amer. Chem. Soc.*, 1935, **57**, 2306, who quote m. p. 61—62°, $[\alpha]_D$ +22° in methanol).

4-*Toluene*-p-*sulphonyl* 2 : 3-*Dimethyl* α-*Methylrhamnoside*.—Removal of the *iso*propylidene residue as described above gave a syrupy *rhamnoside*, $[\alpha]_D$ −73·5° (*c*, 1·4 in chloroform), n_D^{12} 1·5208 (Found: S, 8·9; OMe, 8·0. $C_{14}H_{20}O_7S$ requires S, 9·6; OMe, 9·3%). Methylation with methyl iodide and silver oxide in 3 operations gave a crystalline *ether* (1·9 g.) which, recrystallised from chloroform–light petroleum (b. p. 60—80°), had m. p. 111°, $[\alpha]_D$ −33° (*c*, 2·0 in chloroform) (Found: C, 52·8; H, 6·4; OMe, 25·0. $C_{16}H_{24}O_7S$ requires C, 53·3; H, 6·7; OMe, 25·8%).

2 : 3-*Dimethyl* α-*Methylrhamnoside and* 2 : 3-*Dimethyl Rhamnose*.—Treatment of the above derivative (1 g.) with sodium amalgam as described previously gave 2 : 3-*dimethyl* α-*methylrhamnoside* as a syrup (0·5 g.) which distilled at 110°/0·05 mm. and had n_D^{16} 1·4538, $[\alpha]_D$ −6° (*c*, 2·0 in water) (Found: OMe, 43·2. $C_9H_{18}O_5$ requires OMe, 45·1%). Hydrolysis with sulphuric acid (4%) was complete in 6 hours; 2 : 3-dimethyl rhamnose isolated as above had $[\alpha]_D$ +40° (*c*, 0·7 in water) (Found: OMe, 30·0. Calc. for $C_8H_{16}O_5$: OMe, 32·3%). Treatment of the syrup (0·4 g.) in ethanol (5 c.c.) with aniline (0·4 g.) at 80° for 2 hours followed by evaporation at room temperature gave an *anilide*, m. p. 138—139° (Found: C, 62·3; H, 7·9; N, 4·9; OMe, 21·4. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·2; OMe, 23·2%).

Periodate Oxidation [with A. G. Ross].—Estimation of the acetaldehyde liberated on oxidation with periodate (Cameron, Ross, and Percival, *J. Soc. Chem. Ind.*, 1948, **67**, 161) gave only 5·6% of the theoretical amount.

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56. *Crystalline* 2 : 3-*Dimethyl* α-D-*Xylose.*

By S. K. Chanda, E. E. Percival, and [the Late] E. G. V. Percival.

By the hydrolysis of methylated esparto xylan Hampton, Haworth, and Hirst (*J.*, 1929, 1739) isolated 2 : 3-dimethyl D-xylose as a syrup, and proved its constitution. This sugar has been isolated several times since (Robertson and Speedie, *J.*, 1934, 824; Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289; Chanda, Hirst, and Percival, *J.*, 1951, 1240) but invariably in a syrupy condition : it has now crystallised.

Experimental.—Purification of a specimen prepared from methylated esparto xylan (Chanda *et al.*, *loc. cit.*) by passage, in light petroleum (b. p. 100—120°)–*n*-butanol (7 : 3), saturated with water, through a column of powdered cellulose in the usual way, isolation of the product, and repetition of the operation gave a colourless syrup which crystallised completely when kept for 2 years in a vacuum over phosphoric oxide. The crystals had m. p. 79—80°, $[\alpha]_D^{15}$ +70° (3 minutes), +68° (5 minutes), +61° (8 minutes), +48° (20 minutes), +37° (35 minutes), +23° (14 hours, constant) (*c*, 1·0 in water) (Found : C, 47·2; H, 8·1. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 8·9%).

King's Buildings, University of Edinburgh. [*Received, October* 16*th*, 1951.]

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825. *Synthesis of* 3 : 4- *and* 3 : 5-*Dimethyl Xylose.*

By E. E. Percival and Rolf Zobrist.

3 : 5-Dimethyl D-xylose has been synthesised from 3 : 5-*iso*propylidene 2-toluene-*p*-sulphonyl methyl-D-xyloside by methanolysis of the *iso*propylidene residue, followed by methylation and reductive fission of the toluene-*p*-sulphonyl group. Confirmation of a previous synthesis by Robertson and Speedie (*J.*, 1934, 825) of 3 : 4-dimethyl xylose has been obtained by substitution of position 2 by a benzoyl group, in place of the toluene-*p*-sulphonyl residue in the above synthesis, followed by a similar series of reactions. This led to a partial ring change from furanose to pyranose, and to the production of a mixture of dimethyl methylxylosides from which crystalline 3 : 4-dimethyl β-methylxyloside has been isolated.

Isolation of 3 : 4-dimethyl xylose from the hydrolysis products of a number of methylated polysaccharides has been reported (Mullan and Percival, *J.*, 1940, 1501; Nelson and Percival, *J.*, 1942, 58; James and Smith, *J.*, 1945, 739), and Robertson and Speedie (*J.*, 1934, 825) have recorded a synthesis based on the blocking of position 2 of the xylose molecule by a benzoyl group and the change of a furanose to a pyranose ring during methanolysis of a 3 : 5-*iso*propylidene residue. In view of the possibility of the migration of the benzoyl group during methylation, the presumption of ring change, and the fact that there is still some doubt concerning the authenticity of the 3 : 4-dimethyl xylose derived from natural products we considered it advisable to repeat this synthesis and to attempt to characterise the product.

Robertson and Speedie prepared methyl 3 : 5-*iso*propylidene methyl-D-xyloside and blocked position 2 with a benzoyl group. Removal of the *iso*propylidene residue with 1% methanolic hydrogen chloride, followed by methylation, debenzoylation, and hydrolysis gave a syrupy dimethyl methylxyloside. Proof of the structure was based on the isolation of a syrupy dimethyl xylose phenylosazone and recovery of unchanged material after 3 days in cold methanolic hydrogen chloride, indicating that furanoside formation was prevented by the presence of a methoxyl residue on $C_{(4)}$. These authors also converted the dimethyl xylose into the 1 : 2-dibenzoate. Replacement of the benzoyl group at $C_{(1)}$ by bromine followed by methoxyl and removal of the benzoyl group from $C_{(2)}$ gave crystalline 3 : 4-dimethyl β-methyl-D-xyloside.

A synthesis essentially similar to this has now been carried out. By complete conversion of xylose into its methylfuranoside by the method of Levene, Raymond, and Dillon (*J. Biol. Chem.*, 1932, **95**, 699) and substitution of anhydrous copper sulphate for hydrogen chloride during the condensation with acetone, the yield of 3 : 5-*iso*propylidene methylxyloside was improved from 17·6 to 70%. In order to avoid the danger of acyl migration the toluene-*p*-sulphonyl residue was used to substitute position 2. This gave crystalline 3 : 5-*iso*propylidene 2-toluene-*p*-sulphonyl methylxyloside (overall yield 41%). Removal of the *iso*propylidene residue by methanolysis followed by methylation and reductive fission of the toluene-*p*-sulphonyl residue gave a syrupy dimethyl methylxyloside. The presence of a furanose ring was indicated by the rate of hydrolysis to the free sugar, the reaction being complete in 0·5 hour with 0·1N-sulphuric acid at 100°. After separation from traces of xylose, monomethyl xylose, and 3 : 4-dimethyl xylose on a cellulose column, the product gave a single spot on a paper chromatogram corresponding to that given by an authentic specimen of 3 : 5-dimethyl xylose kindly supplied by Dr. R. G. Laidlaw. Oxidation of 3 : 5-dimethyl xylose, isolated from the column, with bromine water gave a syrupy lactone, the rate of hydrolysis of which was similar to that quoted by Haworth and

Porter (*J.*, 1928, 617) for 3 : 5-dimethyl xylonolactone. Conversion of the 3 : 5-dimethyl xylose into trimethyl methylxyloside, followed by oxidation with nitric acid, esterification, and amide formation gave crystalline 2 : 3-dimethoxysuccindiamide, $[\alpha]_D$ +100° in water. Had Walden inversion occurred on removal of the toluenesulphonyl residue from $C_{(2)}$ the sugar would have been a dimethyl lyxose, which on methylation followed by oxidation would give rise to inactive dimethoxysuccinic acid.

Since only a trace of 3 : 4-dimethyl xylose was formed during this synthesis the presence of the toluenesulphonyl residue on position 2 had clearly prevented ring change during the methanolysis and subsequent methylation. An explanation similar to that advanced by Percival and Percival (*J.*, 1938, 1585) to account for the stability of the glycosidic group in 2 : 4 : 6-trimethyl 3-toluene-*p*-sulphonyl methylgalactoside may be put forward. The close proximity of the toluenesulphonyl residue in the 2 position of the xylose derivatives to the glycosidic hydrogen atom, especially in the β-form, renders the ring completely stable either by mechanical shielding by the large aromatic residue or by formation of a bond between the glycosidic hydrogen atom and the strongly electronegative oxygen atoms of the sulphonyl residue.

Robertson and Speedie's synthesis (*loc. cit.*) was repeated therefore to ascertain the influence of the benzoyl group in the 2-position. The dimethyl methylxyloside prepared in this experiment partly crystallised and the crystals (A) (10·5% of the total yield) were shown to be 3 : 4-dimethyl methylxyloside, by a mixed melting point with an authentic specimen supplied by Dr. J. K. N. Jones. This is in agreement with Robertson and Speedie's results and showed that there had been at least a partial change in ring form from furanose to pyranose. The presence of a pyranose ring in the crystals (A) was proved by the rate of hydrolysis to the dimethyl sugar (see Experimental section). Identity as 3 : 4-dimethyl xylose was confirmed by oxidation with bromine water to crystalline 3 : 4-dimethyl xylonolactone (an authentic specimen was kindly supplied by Dr. J. K. N. Jones). Complete methylation of the crystals (A) gave crystalline 2 : 3 : 4-trimethyl methylxyloside, and oxidation with nitric acid, esterification, and amide formation gave crystalline inactive *xylo*trimethoxyglutardiamide.

The syrup from which the crystals (A) had been removed was hydrolysed with sulphuric acid and the product separated on a cellulose column into 2 : 3 : 4-trimethyl xylose 22·5%, a mixture of dimethyl xyloses 41%, crystalline 2 : 4-dimethyl xylose 5%, and a mixture of monomethyl xylose and xylose 31·5%. Robertson and Speedie (*loc. cit.*) remarked on the presence of methyl benzoate after treatment with methyl-alcoholic hydrogen chloride and the presence of trimethyl methylxyloside after methylation. Separation of the mixture of dimethyl xyloses on the cellulose column was not successful but analysis by quantitative paper chromatography (Hirst, Hough, and Jones, *J.*, 1949, 928) showed 3 : 5- 26%, 3 : 4- 37%, and 2 : 4-dimethyl xylose 23%, and an unidentified portion 14%.

The mechanism of the reaction is difficult to explain since a number of transformations appear to take place simultaneously. That partial transformation from the furanose to the pyranose ring takes place when the benzoyl group occupies position 2 is shown by the isolation of 3 : 4-dimethyl xylose. The most likely explanation is that this occurs during the removal of the *iso*propylidene group through the influence of the methanolic hydrogen chloride. The reaction is complicated, however, by migration of some of the benzoyl substituent to the 3 position, as shown by the isolation of crystalline 2 : 4-dimethyl xylose. A further complication arises from loss of the benzoyl group and the isolation of considerable quantities of 2 : 3 : 4-trimethyl xylose.

Experimental

3 : 5-iso*Propylidene Methyl*-D-*xyloside*.—Xylose (22 g.) was kept at room temperature with methanolic hydrogen chloride (500 c.c.; 0·5%) until the reducing power had dropped to 5% of the initial value (5 hours) (see Levene, Raymond, and Dillon, *loc. cit.*). Neutralisation with silver carbonate, filtration, and evaporation gave a slightly reducing syrup which after extraction with ethyl acetate and removal of the latter gave a non-reducing syrup (19·2 g.). This was converted into the 3 : 5-*iso*propylidene derivative by Percival and Percival's method (*J.*, 1950, 690). The product was a colourless syrup (B) (22·5 g.) which distilled at 110°/0·1

mm. (21·3 g.), and had n_D^{15} 1·4640, $[\alpha]_D^{15}$ −26° (*c*, 0·6 in water) (Found : $COMe_2$, 30·1. Calc. for $C_9H_{16}O_5$: $COMe_2$, 28·4%).

3 : 5-*Dimethyl* 2-*Toluene*-p-*sulphonyl Methyl*-D-*xyloside*.—To the syrup (B) (21·3 g.) in dry pyridine (60 c.c.) was added powdered toluene-*p*-sulphonyl chloride (29 g.). After 2 days at 15° the mixture was poured on ice, giving a crystalline solid (29·1 g.). Recrystallisation from methanol gave 3 : 5-iso*propylidene* 2-*toluene*-p-*sulphonyl methyl*-D-*xyloside* (19·1 g.), m. p. 120°, $[\alpha]_D^{15}$ −45° (*c*, 0·1 in methanol) (Found : C, 54·4; H, 6·0; S, 9·0. $C_{16}H_{22}O_7S$ requires C, 53·6; H, 6·2; S, 8·95%). Treatment of this substance (19·10 g.) with methanolic hydrogen chloride (100 c.c.; 1%) at 70° for 1 hour gave 2-*toluene*-p-*sulphonyl methylxylofuranoside* as a yellow syrup (16·9 g.), n_D^{15} 1·5269, $[\alpha]_D^{15}$ +41° (*c*, 1·2 in methanol). This substance (16·5 g.) was then methylated 3 times with methyl iodide and silver oxide, giving a pale yellow syrup (17·9 g.), n_D^{15} 1·5025. Solution in light petroleum (b. p. 60—80°) (100 c.c.) and extraction with water (4 × 10 c.c.) removed any fully methylated xyloside. After drying (Na_2SO_4), the light petroleum was evaporated, leaving 3 : 5-*dimethyl* 2-*toluene*-p-*sulphonyl methyl*-D-*xyloside* as a colourless oil (C) (15·5 g.), n_D^{18} 1·5061 (Found : OMe, 26·45. $C_{15}H_{22}O_7S$ requires OMe, 26·9%).

3 : 5-*Dimethyl Methylxyloside*.—The substance (C) (15·4 g.) in methanol (300 c.c.)–water (80 c.c.) was reduced with sodium amalgam (150 g.; 4%) with stirring at 35° during 16 hours. After filtration and repeated extraction with chloroform the chloroform extracts were neutralised with carbon dioxide, dried (Na_2SO_4), and evaporated under diminished pressure. Extraction of the residue with ethyl acetate and removal of the solvent gave a syrup which on distillation gave : Fraction 1 (4·3 g.), b. p. 105—115°/0·01 mm., n_D 1·4500 (Found : OMe, 49·3. Calc. for $C_8H_{16}O_5$: OMe, 48·4%), shown to consist of 3 : 5-dimethyl xylose very slightly contaminated with xylose, monomethyl xylose, and 3 : 4-dimethyl xylose; fraction 2 (2·3 g.), b. p. 115—130°/0·01 mm., n_D 1·4553 (Found : OMe, 26·2%), which was purified by extraction with ethyl acetate from aqueous solution. Redistillation at 105—115°/0·1 mm. gave a colourless syrup (1·1 g.), n_D^{15} 1·4512 (Found : OMe, 47·9%).

Characterisation of 3 : 5-*Dimethyl Xylose*.—The 3 : 5-dimethyl methylxyloside (138 mg.), $[\alpha]_D^{15}$ + 102° (*c*, 1·39 in water), was hydrolysed at 100° with 0·1N-sulphuric acid (11 c.c.) until the rotation ($[\alpha]_D^{15}$ +17·5°) was constant (0·5 hour). After neutralisation of the solution with barium carbonate and evaporation to dryness 3 : 5-dimethyl xylose was obtained as a colourless syrup. This was separated from traces of 3 : 4-dimethyl xylose, monomethyl xylose, and xylose on a column of powdered cellulose (Chanda, Hirst, and Percival, *J.*, 1951, 1240). The solvent employed for elution was purified light petroleum (b. p. 100—120°)–*n*-butanol (6 : 4) saturated with water. A colourless syrup was obtained, having n_D 1·4583, $[\alpha]_D$ +23° (*c*, 1·3 in water), +10° (*c*, 1·3 in chloroform). Laidlaw (*J.*, 1952, 2942) reports +25° (*c*, 1·13 in water), +11° (*c*, 0·84 in chloroform). Attempts to prepare a crystalline anilide were unsuccessful, with the exception of once when a minute quantity of crystals, m. p. 126—127°, was obtained. The sugar (0·8 g.) was oxidised to the lactone (0·7 g.), b. p. 110—125°/0·08 mm., $[\alpha]_D$ +75° (4 minutes), +82° (24 minutes), +72° (24 hours), +67° (7 days), +56° (11 days), +27° (33 days, constant) (*c*, 1·34 in water) (cf. Laidlaw, *loc. cit.*; Haworth and Porter, *loc. cit.*; Levene and Raymond, *J. Biol. Chem.*, 1933, **102**, 331).

3 : 5-Dimethyl methylxyloside (0·8 g.) was methylated 3 times with methyl iodide and silver oxide, and a colourless syrup (2 : 3 : 5-trimethyl methylxylose) (0·8 g.) was isolated. This had n_D^{15} 1·4431, $[\alpha]_D^{15}$ +114° (*c*, 0·1 in methanol), +134° (*c*, 0·33 in water) (Found : OMe, 60·6. Calc. for $C_9H_{18}O_6$: OMe, 60·3%). This syrup (0·7 g.) in nitric acid (10 c.c.; *d* 1·4) was heated at 50° until the evolution of brown fumes had ceased (2 hours). The solution was then kept at 95° for 6 hours. The acid was removed by continuous addition and removal of water under diminished pressure. The syrup (0·420 g.) so obtained was esterified by methanolic hydrogen chloride (10 c.c.; 2%) for 6 hours at 65°. After neutralisation and evaporation of the solvent distillation gave a syrupy ester (0·25 g.), b. p. 120—130°/0·1 mm., n_D^{15} 1·4370, $[\alpha]_D^{15}$ +52° (*c*, 0·1 in methanol). Treatment of this ester for 2 days at 0° with methanol (10 c.c.) saturated with ammonia gave crystals of 2 : 3-dimethoxysuccindiamide (18 mg.), m. p. 270° (decomp.), unchanged on admixture with an authentic specimen, $[\alpha]_D$ +100° (*c*, 0·31 in water) (Found: OMe, 35·5; N, 16·2. Calc. for $C_6H_{12}O_4N_2$: OMe, 35·2, N, 15·9%).

2-*Benzoyl* 3 : 4-*Dimethyl Methylxyloside*.—3 : 5-*iso*Propylidene methylxyloside (6·4 g.) was treated with benzoyl chloride by Robertson and Speedie's method (*loc. cit.*). The product was a syrup (8·8 g., 94·5%) which partly crystallised at 0°. The crystals (2-benzoyl 3 : 5-*iso*propylidene methylxyloside) (1·2 g.) had m. p. 86°, $[\alpha]_D^{15}$ +114° (*c*, 0·1 in chloroform) (Found : C, 62·3; H, 6·5; $COMe_2$, 18·65. Calc. for $C_{16}H_{19}O_6$: C, 62·5; H, 6·2; $COMe_2$, 18·8%). A mixture of syrup and crystals (7·6 g.) when treated with methanolic hydrogen chloride as described

for the 2-toluenesulphonyl derivative gave a syrup (6·6 g.), n_D 1·5217, $[\alpha]_D^{15}$ +31° (*c*, 1·3 in chloroform) (Found : OMe, 16·15. Calc. for $C_{13}H_{16}O_6$: OMe, 11·6%), which after 3 methylations with silver oxide and methyl iodide gave a pale yellow syrup (6·4 g.). Solution of the latter in benzene and extraction with water (4 × 25 c.c.) partly removed trimethyl methylxyloside. The purified material (5·2 g., 71%) was debenzoylated by Zemplén's method (*Ber.*, 1929, **62**, 1613), giving a colourless syrup (D) (2·3 g., 68%) which partly crystallised. The crystals (needles ; 0·25 g.) were separated and after recrystallisation from light petroleum (b. p. 60—80°) had m. p. 88—89°, not depressed by an authentic specimen of 3 : 4-dimethyl methylxyloside supplied by Dr. J. K. N. Jones (Found : C, 49·9; H, 8·3; OMe, 48·5. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·4; OMe, 48·45%), $[\alpha]_D^{18}$ −33° (*c*, 2·48 in chloroform), −58° (*c*, 0·47 in water). Robertson and Speedie recorded m. p. 89—90°, $[\alpha]_D$ −82·5° (*c*, 2·0 in chloroform), for 3 : 4-dimethyl β-methylxyloside.

Characterisation of 3 : 4-*Dimethyl Xylose.*—Crystalline 3 : 4-dimethyl methylxyloside (47 mg.), $[\alpha]_D^{15}$ −58° (*c*, 0·47 in water), was hydrolysed with 0·1N-sulphuric acid at 100° for 6·5 hours, by which time the rotation had changed to $[\alpha]_D^{15}$ +22°. After a further 40 minutes' heating with N-sulphuric acid the rotation was $[\alpha]_D^{15}$ +35° (constant). After neutralisation and evaporation of the solvent 3 : 4-dimethyl xylose was obtained as a colourless syrup (35 mg.). This was oxidised by bromine water in the usual way. The product which distilled at 125—135°/0·03 mm. solidified on cooling and was obtained on recrystallisation from light petroleum (b. p. 60—80°) as large needles, m. p. 68°, unchanged on admixture with an authentic sample of 3 : 4-dimethyl xylonolactone.

Crystalline 3 : 4-dimethyl methylxyloside (250 mg.) when twice treated with methyl iodide and silver oxide gave crystalline 2 : 3 : 4-trimethyl methylxyloside which after purification by sublimation (yield 106 mg.) had m. p. 44—45°, $[\alpha]_D^{15}$ −46° (*c*, 1·0 in chloroform). This substance (106 mg.) was oxidised with nitric acid as described previously. Esterification of the product with methanolic hydrogen chloride followed by amide formation with methanolic ammonia gave crystalline *xylo*trimethoxyglutardiamide, m. p. 197°, alone or on admixture with an authentic specimen, $[\alpha]_D$ 0° (*c*, 0·5 in water).

Examination of the Syrup (*D*).—Hydrolysis of the syrup (D), after removal of the crystalline 3 : 4-dimethyl methylxyloside, with N-sulphuric acid at 100° until the rotation was constant ($[\alpha]_D$ +36°), and examination of the product on a paper chromatogram revealed the presence of trimethyl xylose (R_G 0·94), 3 : 5- (R_G 0·52), 3 : 4- (R_G 0·42), and 2 : 4-dimethyl xylose (R_G 0·39), an unidentified sugar (R_G 0·30), and some monomethyl xylose and xylose. This mixture (1·06 g.) was separated on a column of powdered cellulose (Chanda, Hirst, and Percival, *loc. cit.*). The solvent employed for elution was purified light petroleum (b. p. 100—120°)–*n*-butanol (1 : 1) saturated with water. Fraction 1 (0·260 g.) was trimethyl xylose, n_D 1·4560. Fraction 2 (0·423 g.) was a mixture of 3 : 5-, 3 : 4-, and 2 : 4-dimethyl xylose. Fraction 3 (0·052 g.) was crystalline 2 : 4-dimethyl xylose, m. p. 110°. Fraction 4 (0·32 g.) was a mixture of monomethyl xylose and xylose. Quantitative determination of the dimethyl sugars in fraction 2 by the method of Hirst, Hough, and Jones (*loc. cit.*) with benzene–amyl alcohol–ethanol–water (1·7 : 1 : 1 : 0·25) as the eluting solvent indicated the presence of 3 : 5-dimethyl xylose 26%, 3 : 4-dimethyl xylose 37%, 2 : 4-dimethyl xylose 23%, and an unidentified sugar (R_G 0·30) 14·5%.

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113. *The Preparation of* 2 : 3-*Anhydromethyl*-D-*lyxoside from* 2-*Toluene*-p-*sulphonyl Methyl*-D-*xylofuranoside, and Synthesis of* 2-*Methyl* D-*Xylose,* 3-*Methyl* D-*Arabinose, and* 3 : 5-*Dimethyl* D-*Arabinose.*

By E. E. Percival and Rolf Zobrist.

Crystalline 2 : 3-anhydromethyl-D-lyxoside has been prepared; hydrolysis of the anhydro-ring with sodium methoxide led to the isolation and characterisation of crystalline 2-methyl D-xylose and syrupy 3-methyl D-arabinose.

Methylation of the anhydro-compound gave crystalline 5-methyl 2 : 3-anhydromethyl-D-lyxoside. Fission of the anhydro-ring, followed by hydrolysis of the glycosidic methoxyl group, gave only one dimethyl pentose which has been identified as 3 : 5-dimethyl D-arabinose.

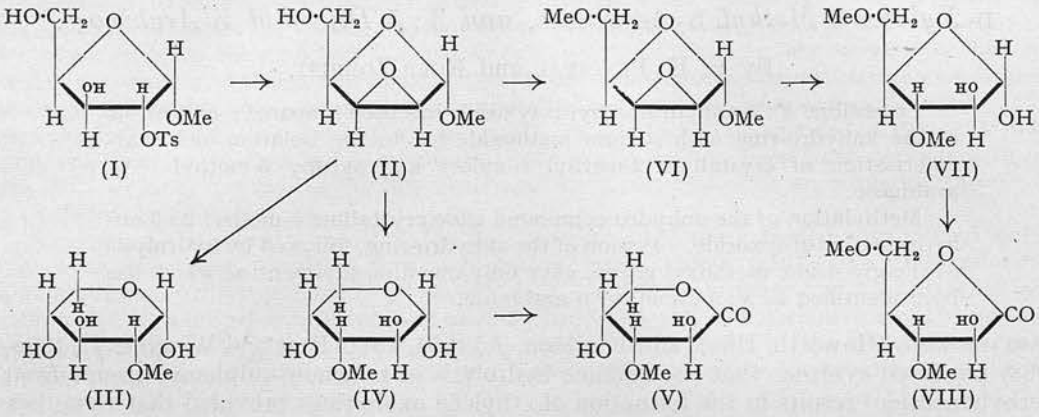
Earlier work (Haworth, Hirst, and Panizzon, *J.*, 1934, 154; Peat and Wiggins, *J.*, 1938, 1088) furnished evidence that the alkaline hydrolysis of toluene-*p*-sulphonyl groups from methylglucosides results in the formation of ethylene oxide rings provided that there is a free hydroxyl group, in the *trans*-position, on the carbon atom adjacent to that carrying the toluenesulphonyl group. If these conditions prevail hydrolysis is accompanied by Walden inversion on the carbon atom which formerly carried the toluenesulphonyl residue. If was also shown by these workers that scission of the oxide ring with sodium methoxide gives two products. The ring can apparently break on either side of the oxygen atom, and at the point of entry of the methoxy-group inversion takes place. It is not always possible to isolate both products, since the proportions vary, that is, the chances of the two oxygen bridges being split are not equal in all such compounds owing to steric factors.

We decided to investigate some of these steric effects and at the same time attempt to synthesise different methylated derivatives of pentoses and methylpentosides. 2-Toluene-*p*-sulphonyl methyl-D-xylofuranoside (I) was used as the starting material. Hydrolysis with alkali gave 2 : 3-anhydromethyl-D-lyxoside (II) and fission of the anhydro-ring gave 2-methyl D-xylose (III) and syrupy 3-methyl D-arabinose (IV) in the ratio of 1 : 2. 2-Methyl xylose had m. p. 134°, not depressed on admixture with an authentic specimen, and on treatment with ethanolic aniline yielded the characteristic anilide (Percival and Willox, *J.*, 1949, 1608). 3-Methyl D-arabinose was a colourless syrup, $[\alpha]_D$ −90° (*c*, 0·84 in H_2O) (initial). Oxidation with bromine water gave a crystalline lactone (V), m. p. 81°, which on treatment with methyl-alcoholic ammonia gave a crystalline amide, m. p. 132°. Hirst, Jones, and Williams (*J.*, 1947, 1062) record m. p. 78° and m. p. 132° for the same derivatives from 3-methyl L-arabinose.

2 : 3-Anhydromethyl-lyxoside was methylated with a view to ascertaining whether a 5-methoxyl group would influence the fission of the oxide ring. Treatment of crystalline 5-methyl 2 : 3-anhydromethyl-lyxoside (VI) with sodium methoxide gave a syrup containing only one dimethyl pentose, 3 : 5-dimethyl D-arabinose, contaminated with traces of trimethyl, monomethyl, and unmethylated pentoses. No evidence for the presence of 2 : 5-dimethyl xylose could be obtained.

Similar treatment of syrupy 2 : 3-anhydromethyl-lyxoside gave an identical syrup. These syrups were combined and freed from traces of impurity by separation on a column of powdered cellulose. Demethylation and chromatographic analysis of the resulting syrup revealed only the presence of arabinose and partially methylated arabinose. No

xylose or methylated xyloses could be found. The pure dimethyl pentose separated from the column was identified as 3 : 5-dimethyl D-arabinose (VII) by oxidation with bromine water to the corresponding dimethyl D-arabonolactone (VIII). This lactone was crystalline (m. p. 74—75°) and its slow rate of mutarotation in aqueous solution ($[\alpha]_D$ +85° changing to +57° in 27 days) indicated that it belonged to the γ-series of arabonolactones (cf. Smith and Cunneen, *J.*, 1948, 1146, who record m. p. 75°, $[\alpha]_D$ −84° changing to −69° in 28 days for 3 : 5-dimethyl L-arabonolactone), and it gave a crystalline phenylhydrazide (Hirst, Jones and Williams, *loc. cit.*). The possibility of the simultaneous formation of 2 : 3- and 2 : 5-anhydromethyl-lyxosides had been envisaged, but no proof of the presence of fission products from the 2 : 5-ring compound could be obtained. It is conceivable, however, that part of the 2-methyl xylose isolated might have been derived from this source.



It is interesting that in the absence of substitution on $C_{(5)}$ hydrolysis of the ethylene oxide compound caused fission of both oxygen bridges and the two expected monomethyl derivatives, 2-methyl D-xylose and 3-methyl D-arabinose, were isolated. However, when a methyl group occupied $C_{(5)}$ a preferential splitting of the ring occurred between the oxygen atom and $C_{(3)}$, and only 3 : 5-dimethyl D-arabinose was obtained. No trace of the other isomer, 2 : 5-dimethyl D-xylose, could be found. Similar results have been obtained with methylated anhydro-derivatives of rhamnose and of fucose (unpublished work).

Experimental

2 : 3-*Anhydromethyl*-D-*lyxoside*.—2-Toluene-*p*-sulphonyl methylxylofuranoside (24 g.) (Percival and Zobrist, *J.*, 1952, 4306) was dissolved in ethanol (400 c.c.) and hydrolysed with 2N-sodium hydroxide at 75° until permanently pink to phenolphthalein (36·3 c.c., 96%). The solvents were removed under diminished pressure. Repeated extraction of the residue with ethyl acetate and removal of the solvent gave a syrup (11 g.) which partly crystallised. The crystals (A) (2·7 g., 23%) were separated and after recrystallisation from benzene had m. p. 81°, $[\alpha]_D^{15}$ +57° (*c*, 1·0 in H_2O) (Found : C, 49·3; H, 6·5; OMe, 20·7. $C_6H_{10}O_4$ requires C, 49·3; H, 6·85; OMe, 21·2%). The residual syrup (B) (8·3 g.) had $[\alpha]_D^{15}$ +4° (*c*, 0·95 in H_2O) (Found : OMe, 20·5%).

Alkaline Hydrolysis of 2 : 3-*Anhydromethyl*-D-*lyxoside*.—Crystalline 2 : 3-anhydromethyl-D-lyxoside (A) (0·1 g.) was heated at 95° for 12 hours with methanol (5 c.c.) containing 4% of sodium. Thereafter, the solution was diluted with water (5 c.c.), neutralised with solid carbon dioxide, and evaporated to dryness. Extraction with chloroform and removal of the solvent gave a colourless syrup. This was hydrolysed at 100° with 0·1N-sulphuric acid (10 c.c.) until no further change in rotation occurred ($[\alpha]_D^{15}$ +63° ⟶ −4·7° in 3 hours). Neutralisation of the solution with barium carbonate and evaporation to dryness gave a colourless syrup (C). Examination on a paper chromatogram with butanol–ethanol–water (4 : 1 : 5) revealed the presence of 2-methyl xylose (R_G 0·38), a second nomomethyl pentose (R_G 0·26), and unmethylated pentose (R_G 0·19).

The residual syrup (B) (6·0 g.) was hydrolysed as described for the crystalline material (A) and, after removal of the glycosidic methoxyl group, gave a pale yellow syrup (D) (4·1 g.).

Comparison on a paper chromatogram of the spots given by this syrup with those given by syrup (C) showed the two syrups to be identical. The above results together with the difference in rotation suggest that the crystals (A) are the α-form of 2 : 3-anhydromethyl-D-lyxoside and that the residual syrup (B) is a mixture of the α- and the β-form of this sugar.

The syrups (C) and (D) (4·0 g.) were combined and separated on a column of powdered cellulose (Chanda, Hirst, and Percival, *J.*, 1951, 1240). Elution was by purified light petroleum (b. p. 100—120°)–*n*-butanol (1 : 9) saturated with water. Fraction 1 (1·05 g.) was crystalline 2-methyl xylose, m. p. 134°, unchanged on admixture with an authentic specimen, $[\alpha]_D^{18}$ −22° (initial), +26° (3 days) (*c*, 0·61 in H_2O) (cf. Percival and Willox, *loc. cit.*) (Found: C, 44·1; H, 7·4; OMe, 18·5. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·3; OMe, 18·9%). Fraction 2 (2·16 g.) was syrupy 3-*methyl* D-*arabinose*, $[\alpha]_D^{15}$ −90° (initial), −43° (3 days) (*c*, 0·84 in H_2O) (Found: OMe, 18·6. $C_6H_{12}O_5$ requires OMe, 18·9%). Hirst, Jones, and Williams (*loc. cit.*) record +110° (*c*, 3·6 in H_2O) for 3-methyl L-arabinose. Aqueous extraction of the column and removal of the water gave a syrup (0·4 g.). Quantitative determination of the sugars present by the method of Hirst, Hough, and Jones (*J.*, 1949, 928) with butanol–ethanol–water (4 : 1 : 5) as the eluting solvent gave arabinose (78%) and xylose (22%).

On being heated with alcoholic aniline (0·15 g. in 5 c.c.) 2-methyl D-xylose (0·1 g.) gave a crystalline aniline compound, m. p. 125—126°, $[\alpha]_D$ +212° (*c*, 0·5 in EtOAc) (cf. Percival and Willox, *loc. cit.*).

Characterisation of 3-*Methyl* D-*Arabinose*.—3-Methyl D-arabinose (0·23 g.) was oxidised to the lactone (0·13 g.), which crystallised immediately and was purified by sublimation in a vacuum; it had m. p. 81°, $[\alpha]_D^{18}$ +99° (5 min.), +104° (30 min.), +80° (3 days), +75° (22 days) (*c*, 2·994 in H_2O) (Found: 43·9; H, 6·7; OMe, 19·1. $C_6H_{10}O_5$ requires C, 44·4; H, 6·2; OMe, 19·1%). With alcoholic ammonia the *lactone* gave 3-methyl D-arabonamide, m. p. 132° (from alcohol and acetone), which gave a positive Weerman test.

5-*Methyl* 2 : 3-*Anhydromethyl-lyxoside*.—Crystalline 2 : 3-anhydromethyl-D-lyxofuranoside (0·60 g.) was methylated 3 times with methyl iodide and silver oxide, and a very volatile crystalline product (0·56 g.) was isolated. This was purified by sublimation in a vacuum and then had m. p. 43° (constant), $[\alpha]_D^{18}$ +60° (*c*, 4·45 in MeOH) (Found: C, 50·8; H, 7·7; OMe, 36·7. $C_7H_{12}O_4$ requires C, 52·5; H, 7·65; OMe, 38·7%).

Methylation of the syrupy 2 : 3-anhydromethyl-lyxofuranoside ($[\alpha]_D$ +4°) (6·0 g.) gave syrupy 5-methyl 2 : 3-anhydromethyl-D-lyxoside (5·5 g.), $[\alpha]_D^{18}$ +24° (*c*, 1·2 in H_2O) (Found: OMe, 39·5%).

Alkaline Hydrolysis of 5-*Methyl* 2 : 3-*Anhydromethyl-D-lyxoside*.—Following the procedure used for the unmethylated material, crystalline 5-methyl 2 : 3-anhydromethyl-lyxoside (0·40 g.) was treated with 4% sodium methoxide solution (25 c.c.). Neutralisation, however, was effected by passage through a column of cation exchange resin (Amberlite I.R.-100H). Evaporation of the solvents gave a colourless syrup (0·26 g.), $[\alpha]_D^{18}$ +64° (*c*, 0·92 in H_2O) (Found: OMe, 47·3. Calc. for $C_8H_{16}O_5$: OMe, 48·45%).

This syrup (0·25 g.) was hydrolysed with N-sulphuric acid at 100° for 1 hour, by which time the rotation had changed to $[\alpha]_D^{20}$ +37° (constant). After neutralisation and evaporation of the solvent a yellow syrup (E) (0·18 g.) was obtained. Chromatographic examination of this syrup with *n*-butanol–ethanol–water (4 : 1 : 5) indicated one main spot (R_G 0·77) and very faint spots corresponding to a trimethyl pentose (R_G 0·94), 2-methyl xylose (R_G 0·38), 3-methyl arabinose (R_G 0·27), and a pentose (R_G 0·19). 2 : 4-Dimethyl xylose, 2-methyl xylose, and xylose were run on the paper as controls.

Hydrolysis of syrupy 5-methyl 2 : 3-anhydromethyl-lyxoside ($[\alpha]_D$ +24°) (5·4 g.) with sodium methoxide gave a yellow syrup (3·8 g.), $[\alpha]_D^{18}$ −17° (*c*, 1·0 in H_2O) (Found: OMe, 47·4. Calc. for $C_8H_{16}O_5$: OMe, 48·45%). Hydrolysis with sulphuric acid gave a syrup (1·8 g.), $[\alpha]_D$ +38°, which revealed the same spots on a paper chromatogram as those given by the syrup (E) obtained from crystalline 5-methyl 2 : 3-anhydromethyl-lyxoside. Purification by passage through a column of powdered cellulose with light petroleum (b. p. 100—120°)–butanol (50 : 50) saturated with water as eluant gave a dimethyl pentose (F) (1·1 g.), $[\alpha]_D^{18}$ +47° (*c*, 0·76 in H_2O; const.) (Found: OMe, 33·9. $C_7H_{14}O_5$ requires OMe, 34·8%).

The purified syrup (F) (0·01 g.) was demethylated by hydriodic acid (1 c.c.) at 100° for 10 minutes. The solution was diluted with water (10 c.c.) neutralised with silver carbonate and filtered. Silver ions were removed by treatment with cation exchange resin (Amberlite I.R.-100H), and the solution was concentrated. Examination on a paper chromatogram with butanol–pyridine–water–benzene (5 : 3 : 3 : 1) as eluant revealed the presence of arabinose and unchanged dimethyl pentose. There was no evidence for the presence of xylose.

Characterisation of 3 : 5-*Dimethyl* D-*Arabinose*.—The sugar (F) (120 mg.) was oxidised with bromine (1 c.c.) in water (5. c.c.) at 20° for 48 hours. The 3 : 5-*dimethyl* D-*arabonolactone* (98 mg.) isolated in the usual way crystallised. The crystals, after recrystallisation from ether, had m. p. 74—75°, $[\alpha]_D^{15}$ +85° (after 15 min., *c*, 0·40 in H_2O), +80° (3 days), +77° (6 days), +57° (27 days) (Found: C, 47·4; H, 6·95; OMe, 34·9. $C_7H_{12}O_5$ requires C, 47·7; H, 6·9; OMe, 35·2%). A portion of the lactone (50 mg.) was heated with alcoholic phenylhydrazine for 2 hours. On cooling, the phenylhydrazide was deposited, as plates, m. p. 144—145°.

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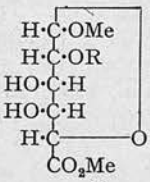
501. *Synthesis of* 2-O-*Methyl- and* 3 : 4-*Di*-O-*methyl*-D-*galacturonic Acid.**

By R. A. Edington and Elizabeth E. Percival.

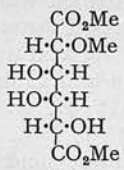
Crystalline methyl (methyl 3 : 4-*O*-*iso*propylidene-α-D-galactosid)uronate (B) has been synthesised from D-galactose. Methylation of (B) gave methyl (methyl 2-*O*-methyl-3 : 4-*O*-*iso*propylidene-α-D-galactosid)uronate (C) which was characterised by isolation of a crystalline amide, by oxidation to dimethyl 2-*O*-methyl-D-galactarate and by formation of the known crystalline diamide thereof. Hydrolysis of (C) gave 2-*O*-methylgalacturonic acid which on appropriate treatment gave the same diamide.

The crystalline 2-toluene-*p*-sulphonyl derivative of (B) was prepared, and removal of the *iso*propylidene residue followed by methylation and reductive fission of the toluene-*p*-sulphonyl group gave methyl 3 : 4-di-*O*-methyl-D-galactosiduronic acid. The methyl ester and its corresponding amide and methylamide were also obtained.

Apart from their intrinsic interest the methyl ethers of galacturonic acid are of great importance in the structural studies of the polysaccharides occurring in plant gums and pectins and in certain bacterial polysaccharides. Methylation followed by hydrolysis of these polysaccharides has led to a number of partially methylated galacturonic acid derivatives (Luckett and Smith, *J.*, 1940, 1106, 1506; Hirst, Hough, and Jones, *J.*, 1949, 3145; Brown, Hirst, and Jones, *J.*, 1949, 1761; Hough and Jones, *J.*, 1950, 1199). In addition G. A. Adams *et al.* (*Canad. J. Res.*, 1950, **28**, *B*, 753; *Canad. J. Chem.*, 1951, **29**, 109) isolated a monomethyl galacturonic acid from the hydrolysis products of the unmethylated polysaccharide from the hemicelluloses of wheat. The present work is concerned with the synthesis of 2-*O*-methyl- and 3 : 4-di-*O*-methyl-galacturonic acid. Methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate (I; R = Me) has been synthesised



(I)



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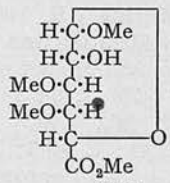

(III)

previously by Jones and Stacey (*J.*, 1947, 1340) from 1 : 2 : 3 : 4-tetra-*O*-acetyl-6-*O*-tritylgalactose, which on removal of the trityl residue and oxidation gave 1 : 2 : 3 : 4-tetra-*O*-acetylgalacturonic acid. Hydrolysis of the acetyl groups and treatment with 1% methanolic hydrogen chloride then gave methyl (methyl α-D-galactosid)uronate (I; R = H). The hydroxyl groups at $C_{(3)}$ and $C_{(4)}$ were blocked by an *iso*propylidene residue and methylation followed by hydrolysis gave methyl (methyl 2-*O*-methyl-D-galactosid)-uronate in approximately 7·5% overall yield. The present synthesis differs in the earlier stages and gives a 20% overall yield. 1 : 2-3 : 4-Di-*O*-*iso*propylidene-D-galactose was oxidised with permanganate, giving crystalline potassium 1 : 2-3 : 4-di-*O*-*iso*propylidene-D-galacturonate monohydrate. This was converted into the crystalline free acid monohydrate which when boiled in dry methanol with cation-exchange resins furnished crystalline methyl (methyl α-D-galactosid)uronate. Although different conditions for the sub-

* For nomenclature, see *J.*, 1952, 5108, especially rules 9, 20, 26, 27, and 28.

stitution and for the removal of the *iso*propylidene residue have been used, the subsequent stages were the same as those employed by Jones and Stacey (*loc. cit.*). In the present experiments the syrupy methyl (methyl 2-*O*-methyl-3 : 4-*O*-*iso*propylidene-α-D-galactopyranosid)uronate was characterised by the isolation of a crystalline amide and by oxidation to dimethyl 2-*O*-methylgalactarate (II). This, on appropriate treatment, gave the known crystalline diamide. Hydrolysis of the *iso*propylidene and glycosidic and ester methoxyl groups from methyl (methyl 2-*O*-methyl-3 : 4-*O*-*iso*propylidene-α-D-galactopyranosid)uronate and oxidation of the resulting 2-*O*-methyl-D-galacturonic acid with bromine water gave the same dimethyl ester which after treatment with methanolic ammonia furnished the above-mentioned crystalline diamide.

Crystalline methyl (methyl 3 : 4-di-*O*-methyl-α-D-galactopyranosid)uronate (III) has been synthesised by blocking position 2 in crystalline methyl (methyl 3 : 4-*O*-*iso*propylidenegalactosid)uronate with a toluene-*p*-sulphonyl group. Hydrolysis of the *iso*propylidene residue followed by methylation gave crystalline methyl (methyl 3 : 4-di-*O*-methyl-2-*O*-toluene-*p*-sulphonylgalactosid)uronate. Reductive fission of the toluene-*p*-sulphonyl group with sodium amalgam and separation from the toluenesulphinic acid and metallic ions by adsorption on ion-exchange resins followed by preferential elution led to the isolation of crystalline methyl 3 : 4-di-*O*-methylgalactosiduronic acid. Treatment with methanolic hydrogen chloride furnished the methyl ester (III) as long needles, and appropriate treatment gave a crystalline amide and methylamide. It was converted into crystalline methyl (methyl-2 : 3 : 4-tri-*O*-methyl-α-D-galactopyranosid)uronate. Hydrolysis of (III) followed by oxidation gave crystalline diethyl 3 : 4-di-*O*-methylgalactarate. The corresponding crystalline dimethyl ester and diamide were isolated.

Experimental

Methyl (*Methyl* α-D-*Galactopyranosid*)*uronate*.—D-Galactose (80·0 g.) was converted into 1 : 2-3 : 4-di-*O*-*iso*propylidene-D-galactose by the method described by Ohle and Berend (*Ber.*, 1925, **58**, 2585). The syrupy product, when distilled in a high vacuum, had b. p. 130—170°/0·03 mm., n_D^{25} 1·4657 (yield, 55·3 g., 76%), $[\alpha]_D^{19}$ −51° (*c*, 1·2 in H_2O) [Found : $COMe_2$, 44·9. Calc. for $C_{12}H_{20}O_6$: $COMe_2$, 44·6%).

Oxidation of this syrup (55·0 g.) by potassium permanganate as described by Ohle and Berend (*loc. cit.*) gave potassium 1 : 2-3 : 4-di-*O*-*iso*propylidene-D-galacturonate monohydrate (48 g., 70%), m. p. 200° (decomp.), $[\alpha]_D^{21}$ −70° (*c*, 2·0 in H_2O) (Found : C, 43·3; H, 5·5; $COMe_2$, 36·0. Calc. for $C_{12}H_{17}O_7K,H_2O$: C, 43·6; H, 5·8; $COMe_2$, 35·2%). 1 : 2-3 : 4-Di-*O*-*iso*propylidene-D-galacturonic acid was obtained by treatment of this (16 g.) with cation-exchange resins (Amberlite I.R. 100-H) (24 g.) in distilled water (200 c.c.) for 6 hr. The resins were removed by filtration and the filtrate was passed through a column (250 × 18 mm.) of the same resin. Removal of part of the water gave the acid as colourless crystals (12·7 g., 91%), m. p. 158°, $[\alpha]_D^{17}$ −79° (*c*, 0·9 in $CHCl_3$) (Niemann and Link, *J. Biol. Chem.*, 1934, **104**, 197, record m. p. 157°, $[\alpha]_D$ −84°) (Found : C, 50·1; H, 6·9; $COMe_2$, 40·0; CO_2, 16·1. Calc. for $C_{12}H_{18}O_7,H_2O$: C, 49·3; H, 6·9; $COMe_2$, 39·8; CO_2, 15·1%).

1 : 2-3 : 4-Di-*O*-*iso*propylidene-D-galacturonic acid monohydrate (13·9 g.) was boiled for 24 hr. with dry methanol (200 c.c.) containing cation-exchange resins (Amberlite I.R. 100-H). Removal of the resins and evaporation gave a colourless syrup which on trituration with ethanol partly crystallised (3·6 g., 32%). Repeated treatment of the mother-liquor with methanol and resins brought the total yield of crystalline methyl (methyl α-D-galactosid)uronate to 7·48 g. (66%), m. p. 145°, $[\alpha]_D^{16}$ +121° (*c*, 1·0 in H_2O) (Jones and Stacey, *loc. cit.*, record m. p. 147°, and Niemann and Link, *loc. cit.*, $[\alpha]_D$ +121°) (Found : C, 39·9; H, 6·6; OMe, 26·5. Calc. for $C_8H_{14}O_7,H_2O$: C, 40·0; H, 6·7; OMe, 25·8%).

Methyl (*Methyl* 3 : 4-O-iso*Propylidene*-α-D-*galactosid*)*uronate*.—Methyl (methyl α-D-galactosid)uronate monohydrate (3·6 g.) was shaken for 120 hr. with dry acetone (250 c.c.) containing acetaldehyde (2 drops) and anhydrous copper sulphate (30 g.). A white solid was obtained which on recrystallisation from light petroleum (b. p. 60—80°) gave colourless needles (3·62 g., 94%), m. p. 113—114°, $[\alpha]_D$ +117° (*c*, 1·2 in H_2O) (Jones and Stacey, *loc. cit.*, record m. p. 107°, $[\alpha]_D^{20}$ +118°) (Found : C, 50·8; H, 6·7; OMe, 23·2. Calc. for $C_{11}H_{18}O_7$: C, 50·4; H, 6·9; OMe, 23·7%).

Methyl (*Methyl* 2-O-*Methyl*-α-D-*galactopyranosid*)*uronate*.—The foregoing *iso*propylidene compound (3·8 g.) was methylated thrice with methyl iodide and silver oxide, and the product

distilled (b. p. 120—130°/0·1 mm.) as a colourless syrup (*A*) (2·70 g., 72%), n_D^{17} 1·4622, $[\alpha]_D^{16}$ +103° (*c*, 0·7 in H_2O), +114° (*c*, 0·8 in MeOH) (Found: C, 52·8; H, 7·4; OMe, 33·3. Calc. for $C_{12}H_{20}O_7$: C, 52·2; H, 7·3; OMe, 33·7%). This was characterised by conversion into the crystalline *amide* by treatment with methanolic ammonia. After recrystallisation from ethanol–light petroleum (b. p. 60—80°) the crystals had m. p. 123—124°, $[\alpha]_D^{19}$ +70° (*c*, 1·1 in H_2O) (Found: C, 49·9; H, 7·1; N, 5·0; OMe, 23·7. $C_{11}H_{19}O_6N$ requires C, 50·6; H, 7·3; N, 5·4; OMe, 23·8%).

Mild hydrolysis of the *iso*propylidene residue was carried out in three ways: (1) A solution of the syrup (*A*) (2·48 g.) in methanol (50 c.c.) containing water (0·20 c.c.) was shaken with dry cation-exchange resins (Amberlite I.R. 100-H) (1·5 g.) for 6 days. Filtration and removal of the solvent gave methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate as a colourless syrup (2·18 g., 98%), n_D^{17} 1·4680, $[\alpha]_D$ +105° (*c*, 0·7 in MeOH), +113° (*c*, 0·8 in H_2O) (Jones and Stacey, *loc. cit.*, record n_D 1·4732, $[\alpha]_D^{20}$ +80°) (Found: C, 45·3; H, 6·9; OMe, 39·7. Calc. for $C_9H_{16}O_7$: C, 45·8; H, 6·8; OMe, 39·4%). (2) Similar treatment of (*A*) for 48 hr. at 50° gave a syrup (98%), $[\alpha]_D$ +97° (*c*, 0·7 in MeOH). (3) Treatment of (*A*) with 0·5% methanolic hydrogen chloride at 70° for 70 min. gave a syrup (98%), n_D^{17} 1·4679, $[\alpha]_D^{17}$ +4·0° (*c*, 1·7 in H_2O) (Brown, Hirst, and Jones, *J.*, 1949, 1761, record $[\alpha]_D$ +21° in H_2O). The derived amide, after recrystallisation from ethanol–ether, had m. p. 174—175°, $[\alpha]_D^{15}$ +60° (*c*, 1·3 in H_2O) (Jones and Stacey, *loc. cit.*, record m. p. 174°, $[\alpha]_D^{18}$ +55° in EtOH) (Found: C, 43·8; H, 6·6; N, 6·8; OMe, 28·4. Calc. for $C_8H_{15}O_6N$: C, 43·4; H, 6·8; N, 6·3; OMe, 28·1%).

Characterisation of Methyl (*Methyl* 2-O-*Methyl*-3: 4-O-iso*propylidene- and* 2-O-*Methyl*-α-D-*galactosid*)*uronate*.—A portion of the syrup (*A*) (0·12 g.) was oxidised with nitric acid (*d* 1·3) by raising the temperature from 40° to 80° during 15 min., and then kept at 80° for 10 min. After removal of the nitric acid by distillation under diminished pressure, with frequent additions of water, the product was esterified by boiling it for 6 hr. with methanolic hydrogen chloride (4%). The resulting ester, dimethyl 2-*O*-methyl-D-galactarate, gave on distillation a syrup (0·07 g.), b. p. 160—180°/0·1 mm., from which a crystalline diamide, m. p. 205° (decomp.), was obtained on treatment with methanolic ammonia for 48 hr. at 0° (Jones and Stacey, *loc. cit.*, record m. p. 200°; Brown, Hirst, and Jones, *loc. cit.*, give m. p. 195° for this product from methylated *Cholla* gum; Hough and Jones, *loc. cit.*, give m. p. 207° for the same derivative from methylated gum from *Sterculia setigera*).

The syrup (*A*) (0·3 g.) was hydrolysed with 0·2N-sulphuric acid at 100° until the rotation was constant ($[\alpha]_D$ +36°; 48 hr.). Neutralisation was effected with barium carbonate and 2-*O*-methyl-D-galacturonic acid was obtained as a colourless syrup ($[\alpha]_D^{18}$ +42°), after elution through a column (160 × 12 mm.) of cation-exchange resin (Amberlite I.R. 120-H). Oxidation with bromine, followed by ester formation, furnished dimethyl 2-*O*-methyl-D-galactarate which had n_D^{15} 1·4640, $[\alpha]_D^{18}$ +34° (*c*, 3·0 in H_2O). The diamide prepared as above had m. p. 205° (decomp.) (Found: C, 37·8; H, 6·2; N, 11·6; OMe, 14·9. Calc. for $C_7H_{14}O_6N_2$: C, 37·8; H, 6·4; N, 12·6; OMe, 14·0%).

Methyl (*Methyl* 3: 4-O-iso*Propylidene*-2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate*.—The above-mentioned *iso*propylidene derivative, m. p. 113—114° (0·931 g.), was dissolved in dry pyridine (20 c.c.) and kept with "Drierite" (12 g.) at 0° for 20 hr. Toluene-*p*-sulphonyl chloride (1·6 g.) in dry pyridine (10 c.c.) was added in small portions during several hours, the mixture being kept at 0°. The solution was then set aside for 24 hr. at 0°, for 24 hr. at 15°, and for 72 hr. at 30° (unless these conditions are observed the yield is much diminished). The mixture was cooled to 0°, the "Drierite" removed by filtration, and water (100 c.c.) cautiously added with constant cooling. *Methyl* (*methyl* 3: 4-O-iso*propylidene*-2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate* (*B*) was deposited as colourless needles (1·02 g.), m. p. 157—158°, $[\alpha]_D^{20}$ +122° (*c*, 1·1 in MeOH), +117° (*c*, 2·2 in $CHCl_3$) (Found: C, 52·0; H, 5·5; S, 8·1; OMe, 14·6. $C_{18}H_{24}O_9S$ requires C, 51·9; H, 5·8; S, 7·8; OMe, 14·9%). After removal of the crystals, extraction of the aqueous filtrate with chloroform and removal of the solvent gave a further yield (0·23 g.) of crystals (total yield, 1·255 g., 85%).

Methyl (*Methyl* 3: 4-*Di*-O-*methyl*-2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate*.—The crystals (*B*) (1·345 g.), dissolved in 1% methanolic hydrogen chloride (130 c.c.), were kept at 30° for 30 hr. After neutralisation with silver carbonate *methyl* (*methyl* 2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate* (*C*) (1·18 g., 97%) was obtained. After recrystallisation from aqueous methanol it had m. p. 71°, $[\alpha]_D^{18}$ +61° (*c*, 1·1 in $CHCl_3$) (Found: C, 45·8; H, 5·7; S, 8·4; OMe, 15·2. $C_{15}H_{20}O_9S,H_2O$ requires C, 45·7; H, 5·6; S, 8·1; OMe, 15·7%). Methanolic anhydrous ammonia quantitatively converted the ester into the *amide* which crystallised on removal of the solvent. The amide after recrystallisation from methanol had m. p. 94—95°, $[\alpha]_D^{18}$ +67°

(*c*, 0·5 in $CHCl_3$) (Found: C, 45·1; H, 5·6; N, 3·5; S, 8·1; OMe, 8·2. $C_{14}H_{19}O_8NS,H_2O$ requires C, 44·3; H, 5·6; N, 3·7; S, 8·4; OMe, 8·2%).

The product (*C*) (1·18 g.) was methylated four times with methyl iodide and silver oxide. The resultant *dimethyl ether* (1·21 g., 96%) was dissolved in warm ethanol and crystallisation induced by the addition of water. After recrystallisation from aqueous methanol it had m. p. 83°, n_D^{20} 1·4900, $[\alpha]_D^{17}$ +82° (*c*, 1·1 in $CHCl_3$), +88° (*c*, 2·4 in EtOH) (Found: C, 50·2; H, 5·8; OMe, 29·5; S, 8·1. $C_{17}H_{24}O_9S$ requires C, 50·5; H, 6·0; OMe, 30·7; S, 7·9%).

Methyl 3 : 4-*Di*-O-*methyl*-α-D-*galactosiduronic Acid*.—Anion-exchange resin (Amberlite I.R.A. 400-OH) was packed into four short glass tubes (60 × 10 mm.) which were arranged alternately with three similar tubes containing cation-exchange resin (Amberlite I.R. 120-H) to form a column, and the whole washed with distilled water (300 c.c.), with ethanol (200 c.c.), and with methanol (200 c.c.).

Methyl (methyl 3 : 4-di-*O*-methyl-2-*O*-toluene-*p*-sulphonyl-α-D-galactosid)uronate (1·2 g.), dissolved in methanol (20 c.c.), was stirred at room temperature with 0·25N-aqueous sodium hydroxide (12 c.c.) during 30 min.; thereafter sodium amalgam (4%; 15 g.) was added during 6 hr. with continuous stirring. The mixture was stirred for a further 18 hr. and then the solids were removed by filtration and washed with methanol. After treatment with solid carbon dioxide the combined filtrates were evaporated to dryness and a white solid was obtained which was repeatedly extracted with dry methanol under reflux. The cooled methanolic extracts (200 c.c.) were passed through a column, prepared as described above, 3—4 c.c. of eluate being collected during a minute, and, after complete elution, the eluate was recycled through the column which was finally washed with methanol (100 c.c.). Removal of the solvent from the combined eluate and washings gave a syrup (0·03 g.) which was discarded. The column was dismantled and the portions containing the anion-exchange resin reassembled and eluted by slow passage (60 hr.) of 2% formic acid in methanol (500 c.c.). Removal of solvent from the eluate, under reduced pressure, gave *methyl* 3 : 4-*di*-O-*methyl*-α-D-*galactosiduronic acid* as a colourless syrup (0·613 g., 87%) which crystallised spontaneously. Recrystallised from ethanol–light petroleum (b. p. 60—80°) it had m. p. 154—155°, $[\alpha]_D^{15}$ +158° (*c*, 1·3 in $CHCl_3$), +156° (*c*, 1·3 in MeOH), +163° (*c*, 1·3 in H_2O) (Found: C, 46·4; H, 7·0; OMe, 38·4. $C_9H_{16}O_7$ requires C, 45·8; H, 6·8; OMe, 39·4%).

Methyl (*Methyl* 3 : 4-*Di*-O-*methyl*-α-D-*galactosid*)*uronate*.—Methyl 3 : 4-di-*O*-methyl-α-D-galactosiduronic acid (0·375 g.), dissolved in methanolic hydrogen chloride (1%; 32 c.c.), was kept at 30° for 48 hr. The solution was neutralised with silver carbonate, and the filtrate after evaporation to dryness at 40°/15 mm. furnished a crystalline ester. Recrystallisation from light petroleum (b. p. 60—80°) gave needles of *methyl* (*methyl* 3 : 4-*di*-O-*methyl*-α-D-*galactosid*)-*uronate* (0·378 g., 95%), m. p. 113—114°, $[\alpha]_D^{16}$ +165° (*c*, 0·4 in $CHCl_3$) (Found: C, 48·0; H, 7·1; OMe, 49·8. $C_{10}H_{18}O_7$ requires C, 48·0; H, 7·25; OMe, 49·6%). The crystalline amide and *methylamide* were prepared by treating the ester, in the usual manner, with methanolic ammonia, and with methanolic methylamine respectively. The amide, after trituration with ethanol, had m. p. 130—131°, $[\alpha]_D^{17}$ +108° (*c*, 1·1 in EtOH). The methylamide, obtained as prisms on recrystallisation from acetone, had m. p. 205°, $[\alpha]_D^{15}$ +116° (*c*, 0·6 in H_2O) (Found: C, 48·5; H, 7·5; N, 5·8; OMe, 38·8. $C_{10}H_{19}O_6N$ requires C, 48·2; H, 7·7; N, 5·6; OMe, 37·4%).

The ester (0·018 g.) was methylated twice with methyl iodide and silver oxide, and crystalline methyl (methyl 2 : 3 : 4-tri-*O*-methyl-α-D-galactopyranosid)uronate (0·019 g.) was obtained. It had m. p. 71—72° (after sublimation *in vacuo*) alone and admixed with an authentic specimen.

Diethyl 3 : 4-*Di*-O-*methylgalactarate*.—The foregoing dimethyl ether ester (0·294 g.) was hydrolysed at 100° with sulphuric acid (20 c.c., 0·2N), the rotations observed being $[\alpha]_D$ +160° (0 hr.), +118° (3·5 hr.), +112° (6·5 hr.), +100° (24 hr.), +90° (31 hr.), +84° (48 hr., const.). The solution was neutralised with barium carbonate and filtered through a well-washed bed of charcoal–"Filter Cel," and barium ions were removed by passage of the filtrate through a column (300 × 10 mm.) of Amberlite (I.R. 120-H) ion-exchange resin. The clear eluate was concentrated to a colourless syrup, n_D^{11} 1·4615, $[\alpha]_D^{16}$ +37° (*c*, 1·2 in EtOH), +93° (*c*, 1·3 in H_2O). The syrup (0·22 g.) in water (15 c.c.) was oxidised with bromine (2 c.c.) at 40°, the rotations observed being $[\alpha]_D$ +93° (0 hr.), 80° (1 day), 60° (3 days), 23° (5 days), 15° (8 days, constant). After removal of the water and hydrobromic acid under diminished pressure with frequent addition of ethanol the syrupy residue was heated in hydrochloric acid (10 c.c.; 0·2N) at 100° for 50 hr. by which time the rotation had fallen to +8°. Further treatment with bromine (2 c.c.) for 3 days at 40° gave a solution with $[\alpha]_D$ ±0°. Removal of the water and hydrobromic acid as above gave crystalline *diethyl* 3 : 4-*di*-O-*methylgalactarate*. Recrystallisation from

aqueous acetone gave flat plates, m. p. 148—149°, $[\alpha]_D$ ±0° [Found: C, 49·4; H, 7·4; OR (as OMe), 42·2. $C_{12}H_{22}O_8$ requires C, 49·0; H, 7·5; OR (as OMe), 42·2%]. Treatment with methanolic hydrogen chloride (1·5%) at 60° for 16 hr. gave a crystalline product. Recrystallisation from acetone–light petroleum (b. p. 60—80°) furnished *dimethyl* 3 : 4-*di*-O-*methylgalactarate* as needles, m. p. 172—173°, $[\alpha]_D$ ±0° (Found: 44·8; H, 6·7. $C_{10}H_{18}O_8$ requires C, 45·1; H, 6·8%). The derived *diamide*, after recrystallisation from methanol, had m. p. 230° (decomp.), $[\alpha]_D$ ±0° (Found: N, 11·9. $C_8H_{16}O_6N_2$ requires N, 11·9%).

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632. *Syntheses of Methyl Ethers of Fructose.*

By E. L. Hirst, W. E. A. Mitchell, Elizabeth E. Percival, and (the late) E. G. V. Percival.

3 : 4 : 6-, 1 : 4 : 6-, 1 : 3 : 4-, and 1 : 4 : 5-tri-*O*-methyl-, 3 : 4- and 4 : 5-di-*O*-methyl-, and 4-*O*-methyl-D-fructoses have been synthesised from one of the crystalline compounds 2 : 3-4 : 5- or 1 : 2-4 : 5-di-*O*-*iso*propylidene-D-fructose or from 2 : 3-*O*-*iso*propylidene-1 : 6-di-*O*-toluene-*p*-sulphonyl-D-fructose. The constitutions of these three *iso*propylidene derivatives are well established.

Three crystalline substances whose constitutions have been established previously have been used as starting materials in this work. 1 : 2-4 : 5-, 2 : 3-4 : 5-Di-*O*-*iso*propylidene-D-fructose (D. J. Bell, *J.*, 1947, 1463) and 2 : 3-*O*-*iso*propylidene-1 : 6-di-*O*-toluene-*p*-sulphonyl-D-fructose (Morgan and Reichstein, *Helv. Chim. Acta*, 1938, **21**, 1023).

Montgomery (*J. Amer. Chem. Soc.*, 1934, **56**, 419) synthesised 3 : 4 : 6-tri-*O*-methylfructose from crystalline 2 : 3-4 : 5-di-*O*-*iso*propylidenefructose by the formation of the 1-benzoyl derivative. In the present synthesis treatment of 2 : 3-4 : 5-di-*O*-*iso*propylidene-D-fructose with toluene-*p*-sulphonyl chloride gave crystalline 2 : 3-4 : 5-di-*O*-*iso*propylidene-1-*O*-toluene-*p*-sulphonyl-D-fructose. Removal of the *iso*propylidene residue followed by conversion into the fructofuranoside led to the isolation of a non-reducing syrup which partly crystallised. Methylation, removal of the toluene-*p*-sulphonyl residue, and hydrolysis gave 3 : 4 : 6-tri-*O*-methylfructose which was characterised by conversion into the crystalline methyl 3 : 4 : 6-tri-*O*-methyl-D-fructuronamide and also by oxidation to crystalline 2 : 3 : 5-tri-*O*-methyl-D-arabonolactone.

1 : 4 : 6-Tri-*O*-methylfructose was synthesised by Montgomery (*loc. cit.*), but only the rotation in chloroform was recorded. In the present work this trimethyl ether has been synthesised from 1 : 2-4 : 5-di-*O*-*iso*propylidenefructose by blocking position 3 with a toluene-*p*-sulphonyl residue. Removal of the *iso*propylidene group and subsequent glycoside formation and methylation followed by reductive fission of the toluene-*p*-sulphonyl group and hydrolysis gave 1 : 4 : 6-tri-*O*-methylfructose as a chromatographically pure syrup.

An alternative synthesis was achieved from crystalline 2 : 3-*O*-*iso*propylidenefructose, obtained by the removal of the toluene-*p*-sulphonyl residues from 2 : 3-*O*-*iso*propylidene-1 : 6-di-*O*-toluene-*p*-sulphonylfructose. Methylation and hydrolysis gave 1 : 4 : 6-tri-*O*-methyl-D-fructose as a chromatographically pure, mobile syrup, identical with the 1 : 4 : 6-tri-*O*-methylfructose obtained from the former synthesis. 4 : 6-Di-*O*-methylfructuronic acid was obtained on oxidation.

Synthesis of 1 : 3 : 4-tri-*O*-methylfructose was achieved by the conversion of methyl 1-*O*-toluene-*p*-sulphonyl-D-fructofuranoside into the 6-*O*-trityl derivative. Removal of the toluene-*p*-sulphonyl residue followed by methylation gave methyl 1 : 3 : 4-tri-*O*-methyl-6-*O*-tritylfructoside. Hydrolysis of the trityl and glycosidic methoxyl groups gave 1 : 3 : 4-tri-*O*-methylfructose as a syrup which partly crystallised. The mother liquors were freed from 1 : 3 : 4 : 6-tetra-*O*-methylfructose by passage through silica gel (Bell and Palmer, *J.*, 1949, 2522).

Neither the isolation nor the synthesis of 1 : 4 : 5-trimethylfructose has been reported previously. Preferential hydrolysis of 2 : 3-4 : 5-di-*O*-*iso*propylidene-D-fructose gave crude 2 : 3-*O*-*iso*propylidene-D-fructopyranose. This was purified by conversion into the crystalline 1 : 4 : 5-tri-*O*-acetyl-2 : 3-*O*-*iso*propylidene-D-fructose. Hydrolysis of the acetyl groups followed by methylation and removal of the *iso*propylidene residue gave 1 : 4 : 5-tri-*O*-methyl-D-fructose, as a mobile syrup.

Macdonald and Jackson (*J. Res. Nat. Bur. Stand.*, 1940, **24**, 181) prepared syrupy 3 : 4-dimethylfructose from di-D-fructofuranose 2 : 1′-2′ : 1-di-anhydride and reported the isolation of a difficultly crystallisable osazone, m. p. 126°, but no other data or constants

were recorded. In the present work methyl 1-*O*-toluene-*p*-sulphonyl-6-*O*-trityl-D-fructoside was methylated and the product on reduction, detritylation, and hydrolysis gave a mixture of 3 : 4-di-*O*-methyl- and 3 : 4 : 6-tri-*O*-methyl-D-fructose, which was separated on cellulose.

3 : 4-Di-*O*-methylfructose has also been synthesised by the removal of the *iso*propylidene residue from 2 : 3-*O*-*iso*propylidene-1 : 6-di-*O*-toluene-*p*-sulphonyl-D-fructose with methanolic hydrogen chloride followed by methylation of the derived glycoside. Reduction and hydrolysis gave 3 : 4-di-*O*-methyl-D-fructose which was again purified from traces of trimethylfructose by separation on cellulose. The constants of the 3 : 4-di-*O*-methylfructose from the two syntheses were in good agreement and the two syrups were chromatographically identical. 3 : 4-Di-*O*-methylfructose was characterised by oxidation and conversion of the product into crystalline (−)-dimethoxysuccinamide and the bismethylamide.

No previous record of the synthesis of 4 : 5-di-*O*-methylfructose could be found. Crystalline 1 : 2-*O*-*iso*propylidene-3-*O*-toluene-*p*-sulphonyl-D-fructose was prepared from the 1 : 2-4 : 5-di-*O*-*iso*propylidene-3-*O*-toluene-*p*-sulphonyl derivative by preferential hydrolysis of the *iso*propylidene residue (Ohle and Just, *Ber.*, 1935, **68**, 601). Methylation followed by reduction gave crystalline 4 : 5-di-*O*-methyl-1 : 2-*O*-*iso*propylidenefructose, and subsequent hydrolysis gave crystalline 4 : 5-di-*O*-methylfructose. A crystalline 2 : 5-dichlorophenylhydrazone and a phenylosazone were prepared.

4-*O*-Methylfructose has been isolated previously from di-D-fructofuranose 2 : 3′-2′ : 1-dianhydride (Macdonald and Jackson, *loc. cit.*) and was identified by the formation of a crystalline osazone, m. p. 156°. In the present work methylation of crystalline 2 : 3-*O*-*iso*propylidene-1 : 6-di-*O*-toluene-*p*-sulphonyl-D-fructose gave crystalline 4-*O*-methyl-2 : 3-*O*-*iso*propylidene-1 : 6-di-*O*-toluene-*p*-sulphonyl-D-fructose. Reduction and hydrolysis led to the isolation of chromatographically pure syrupy 4-*O*-methylfructose. A crystalline osazone, m. p. 158°, identical with 4-*O*-methylglucosazone, was prepared. 4-*O*-Methylfructose was characterised by the same method as for 3 : 4-di-*O*-methylfructose; crystalline-D$_g$-*threo*-2-hydroxy-3-methoxysuccinamide and the bismethylamide were isolated.

Experimental

All solvents were removed under reduced pressure and below 50°. The solvents used for elution, unless otherwise stated, were (I) *n*-butanol–ethanol–water (4 : 1 : 5) and (II) benzene–ethanol–water (167 : 47 : 15). Amberlite resins were used throughout.

3 : 4 : 6-*Tri*-O-*methylfructose*.—2 : 3-4 : 5-Di-*O*-*iso*propylidene-D-fructose, prepared from D-fructose (100 g.) according to the conditions used by Bell (*loc. cit.*), formed transparent needles (105 g., 73%) [from light petroleum (b. p. 60—80°)], m. p. 96—97°, $[\alpha]_D^{16}$ −34·1° (*c*, 5·6 in $CHCl_3$). To a solution of the crystals (82 g.) in dry pyridine (500 c.c.) finely powdered toluene-*p*-sulphonyl chloride (140 g.) was added during 2 hr. The 2 : 3-4 : 5-di-*O*-*iso*propylidene-1-*O*-toluene-*p*-sulphonyl-D-fructose (*A*) (115 g., 88%), isolated in the usual way (Percival and Zobrist, *J.*, 1952, 4306), had m. p. 82°, $[\alpha]_D^{16}$ −26° (*c*, 1·8 in MeOH) (cf. Ohle and Koller, *Ber.*, 1924, **57**, 1566, who record m. p. 83°, $[\alpha]_D^{19}$ −27° in EtOH) (Found: C, 54·7; H, 6·1; S, 7·5. Calc. for $C_{19}H_{26}O_8S$: C, 55·05; H, 6·3; S, 7·8%).

The crystals (*A*) (30 g.) were dissolved in ethanol (50 c.c.), and water (50 c.c.) was added until the solution became slightly turbid. Cation-exchange resin (40 g.) (1R-100 H) was added and the mixture refluxed with vigorous stirring (Glen, Myers, and Grant, *J.*, 1951, 2570) to complete dissolution (20 hr.). Filtration and neutralisation of the filtrate by shaking it with anion-exchange resin (IR-4B) (5 g.) for 2 hr. and removing the solvent gave 1-O-*toluene*-p-*sulphonyl*-D-*fructose* (*B*) (22·6 g., 93%), $[\alpha]_D^{17}$ −23° (*c*, 0·8 in MeOH) (Found: S, 9·1. $C_{13}H_{18}O_8S$ requires S, 9·5%). The syrup (*B*) (13·8 g.), dissolved in methanolic hydrogen chloride (1%; 500 c.c.), was kept at 25° for 100 hr., the maximum rotation ($[\alpha]_D$ +3°) having then been attained. (Further treatment led to a decrease in rotation and the formation of the pyranoside.) Isolation in the usual manner gave a *methyl* 1-O-*toluene*-p-*sulphonylfructoside*, a syrup (*C*) (9·0 g., 63%), $[\alpha]_D^{18}$ +16° (*c*, 1·5 in MeOH) (Found: S, 8·6. $C_{14}H_{20}O_8S$ requires S, 9·2%). When kept this syrup partly crystallised; the crystals had m.p. 79—81°, $[\alpha]_D^{18}$ +15·2° (*c*, 1·0 in MeOH) (Found: C, 48·8; H, 5·6; S, 8·7. $C_{14}H_{20}O_8S$ requires C, 48·3; H, 5·8; S, 9·2%). Fivefold methylation of this derivative (9·0 g.) with methyl iodide and silver oxide gave *methyl* 3 : 4 : 6-*tri*-O-*methyl*-1-O-*toluene*-p-*sulphonylfructoside* (8·3 g., 82%), $[\alpha]_D^{18}$ +32° (*c*, 1·2 in MeOH) (Found: OMe, 31·8; S, 8·4.

$C_{17}H_{26}O_8S$ requires OMe, 32·8; S, 8·5%). This syrup (8·3 g.), dissolved in methanol (140 c.c.), and water (75 c.c.) added to cause slight turbidity, was treated with sodium amalgam (4%; 200 g.) at 40—45° with vigorous stirring for 17 hr. The filtered solution was extracted with chloroform, and the chloroform extract dried (Na_2SO_4), saturated with carbon dioxide (to pH 7), and filtered. Evaporation and distillation at 120°/0·03 mm. gave methyl 3 : 4 : 6-tri-*O*-methyl-D-fructoside as a mobile syrup (4·55 g.), $[\alpha]_D^{19}$ +67·6° (*c*, 1·05 in MeOH) (Found: OMe, 51·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·8%). This furanoside (4·45 g.) was hydrolysed to 3 : 4 : 6-tri-*O*-methylfructose by 0·1N-sulphuric acid (250 c.c.) at 95° for 2·5 hr. (whereafter the rotation remained constant). A syrup (*D*) (3·91 g.) was obtained having n_D^{18} 1·4661, $[\alpha]_D^{18}$ +27° (initial); +29° (24 hr.) (*c*, 1·4 in H_2O) (Found: C, 47·9; H, 8·1; OMe, 41·1. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2; OMe, 41·9%). Chromatographic analysis produced a single spot, R_G 0·89 in solvent (I) and 0·75 in solvent (II).

Characterisation of 3 : 4 : 6-*Trimethyl*-D-*fructose*.—(a) *Oxidation with nitric acid followed by barium permanganate*. The syrup (*D*) (0·201 g.) was oxidised with nitric acid (2·5 c.c.; *d* 1·42) (Mullan and Percival, *J.*, 1940, 1505). The syrup (*E*) finally obtained was dried with anhydrous methanol and esterified with boiling methanolic hydrogen chloride (4%; 7 c.c.) for 17 hr. The derived amide (0·048 g.) had m. p. 98—100° (from ether) and showed no depression on admixture with authentic methyl 3 : 4 : 6-tri-*O*-methyl-D-fructuronamide (Found: C, 47·8; H, 7·4; N, 5·5. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·7; N, 5·6%).

3 : 4 : 6-Tri-*O*-methyl-D-fructuronic acid (*E*) (0·182 g.) was oxidised with barium permanganate according to the conditions described by Avery, Haworth, and Hirst (*J.*, 1927, 2317). The light petroleum extracts yielded a syrup which after distillation at 90°/0·003 mm. gave crystals (0·052 g.), m. p. 30—31°, $[\alpha]_D^{18}$ +44·8° ⟶ 24·0° (24 days) (*c*, 0·96 in H_2O) (Avery, Haworth, and Hirst, *loc. cit.*, record m. p. 32—33°, $[\alpha]_D$ +44·5° ⟶ 25·5°, for 2 : 3 : 5-tri-*O*-methylarabonolactone).

(b) *Oxidation with periodate*. To a solution of 3 : 4 : 6-tri-*O*-methylfructose (*D*) (0·19 g.) in water (10 c.c.), sodium metaperiodate solution (0·3M; 15 c.c.) was added and the solution set aside in the dark at room temperature for 72 hr. The excess of periodate was destroyed by the addition of ethylene glycol (5 c.c.), and the solution extracted thrice with chloroform (80 c.c.). The chloroform extracts were evaporated and the residue extracted with acetone, which gave a syrup that was repeatedly extracted with hot light petroleum (b. p. 60—80°). Removal of the light petroleum and distillation of the syrup gave material (0·096 g., 60%), m. p. 30—31° undepressed on admixture with 2 : 3 : 5-tri-*O*-methylarabonolactone, isolated from the previous oxidation with permanganate.

1 : 4 : 6-*Tri*-O-*methyl*-D-*fructose*.—(*a*) Fructose (60 g.) was condensed with acetone (600 c.c.) in the presence of sulphuric acid (0·3%) (cf. Bell, *loc. cit.*), giving 1 : 2-4 : 5-di-*O*-*iso*propylidenefructose (67·5 g., 78%), m. p. 118—119°, $[\alpha]_D^{16}$ −147° (*c*, 1·5 in $CHCl_3$). This product (58 g.) was converted into the 3-*O*-toluene-*p*-sulphonyl derivative as described above for the 1-*O*-tosyl derivative. The product (*F*) (68 g., 74%) crystallised from aqueous methanol and then from light petroleum (b. p. 60—80°) had m. p. 97—98°, $[\alpha]_D^{17}$ −161° (*c*, 1·0 in MeOH) (Found: C, 54·3; H, 6·0; S, 7·3. Calc. for $C_{19}H_{26}O_8$: C, 55·1; H, 6·3; S, 7·8%). The *iso*propylidene residues were removed and the glycoside formed by refluxing it (60 g.) with methanolic hydrogen chloride (1·5%; 500 c.c.). The rotation changed: −70·9° (10 min.); +9·1° (1 hr.); +10·5° (2 hr.). The hydrochloric acid concentration was adjusted to 0·5N, and the glycoside was then completely hydrolysed at 85° during 3 hr. Amorphous 3-O-*toluene*-p-*sulphonyl*-D-*fructose* (*G*) (42 g., 87%), $[\alpha]_D^{18}$ −36·5° (*c*, 1·7 in MeOH), was obtained (Found: C, 46·1; H, 5·6; S, 8·6. $C_{13}H_{18}O_8S$ requires C, 46·7; H, 5·4; S, 9·6%).

This compound (*G*) (10·5 g.) in methanolic hydrogen chloride was set aside at 35° for 17 hr., maximum rotation then being attained ($[\alpha]_D$ +1·7°). The mixture was neutralised with diazomethane, the solvent removed, and the residual syrup extracted with ether. Removal of solvent then gave the *furanoside* as a syrup (8·0 g., 73%), $[\alpha]_D^{19}$ +14° (*c*, 2·0 in MeOH) (Found: S, 8·8. $C_{14}H_{20}O_8S$ requires S, 9·2%).

The glycoside (8 g.), dissolved in the minimum quantity of methanol (10 c.c.), was methylated four times with methyl iodide and silver oxide; *methyl* 1 : 4 : 6-*tri*-O-*methyl*-3-O-*toluene*-p-*sulphonyl*-D-*fructoside*, formed a syrup (6·3 g., 70%), $[\alpha]_D^{18}$ +28° (*c*, 1·5 in MeOH) (Found: OMe, 32·1; S, 8·3. $C_{17}H_{26}O_8S$ requires OMe, 32·8; S, 8·5%). Treatment of this product (6·3 g.) with sodium amalgam as described above for (*C*) gave methyl 1 : 4 : 6-tri-*O*-methyl-D-fructoside (3·7 g., 97%) which after distillation at 100°/0·007 mm. had $[\alpha]_D^{18}$ +47° (*c*, 1·0 in MeOH) (Found: OMe, 47·2. Calc. for $C_{10}H_{20}O_6$: OMe, 52·8%). The lesser methylated derivatives were removed by dissolving the syrup (3·03 g.) in water (150 c.c.) and extracting

with chloroform (5 × 150 c.c.) (Macdonald, *J. Amer. Chem. Soc.*, 1935, **57**, 772). The combined chloroform extracts were dried (Na_2SO_4) and evaporated. A straw-coloured syrup (*H*) (2·8 g.), $[\alpha]_D^{17}$ +44° (*c*, 1·0 in MeOH), was obtained (Found : OMe, 51·0%).

Attempts to detect any methyl 1 : 4 : 5-tri-*O*-methylfructopyranoside in syrup (*H*) by graded hydrolysis of a portion with hydrobromic acid (0·04N) at 100° (Ford and Peat, *J.*, 1941, 856) were unsuccessful. Preferential formation of the furanoside from the hydrolysed syrup was also attempted. The hydrolysed syrup (0·1 g.) dissolved in methanolic hydrogen chloride (0·5%) was set aside at 18°; the rotation changed from +19·2° (3 min.) to +38·3° (4·5 hr., constant). Since, under these conditions pyranoside formation is unlikely, extraction of an aqueous solution of this fructoside with chloroform should separate any unchanged 1 : 4 : 5-tri-*O*-methylfructose. No difference in rotation was observed in the extracts, from which it was concluded that the sample was pure 1 : 4 : 6-tri-*O*-methylfructose.

The main bulk of (*H*) (2·42 g.) was hydrolysed with sulphuric acid (150 c.c.; 0·1N) at 95° for 2 hr. After purification with charcoal and Filter Cel there was obtained a syrup (1·82 g., 80%), n_D^{18} 1·4643, $[\alpha]_D^{18}$ +19° (*c*, 1·6 in $CHCl_3$), +30° (*c*, 1·13 in H_2O) (Found : C, 47·9; H, 8·1; OMe, 40·9. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2; OMe, 41·9%). Chromatographic analysis gave a single spot R_G 0·91 in solvent (I) and 0·79 in solvent (II) compared with tetramethylglucose R_G 1·0 as control. Attempts to prepare a crystalline phenylosazone were unsuccessful.

(*b*) Crystalline 2 : 3-*O*-*iso*propylidene-D-fructofuranose (7·7 g.) prepared according to Morgan and Reichstein's method (*loc. cit.*) was subjected to four Purdie methylations; 1 : 4 : 6-*tri*-O-*methyl*-2 : 3-O-iso*propylidenefructose* (*J*) was obtained as a chromatographically pure (naphtharesorcinol spray), mobile syrup (8·2 g., 90%), $[\alpha]_D^{18}$ +10° (*c*, 1·3 in EtOH) (Found : OMe, 35·1. $C_{12}H_{22}O_6$ requires OMe, 35·5%). Removal of the *iso*propylidene group with 0·1N-sulphuric acid gave chromatographically pure 1 : 4 : 6-tri-*O*-methylfructose as a mobile syrup (6·0 g. from 8·0 g.; 89%), n_D^{18} 1·4638, $[\alpha]_D^{19}$ +24° (initial) ⟶ +27° (2·5 hr.) (*c*, 1·2 in H_2O).

Characterisation of 1 : 4 : 6-*Tri*-O-*methyl*-D-*fructose*.—Syrupy 1 : 4 : 6-tri-*O*-methyl-D-fructose (1·2 g.) synthesised by both methods was oxidised with nitric acid, under the conditions described for 3 : 4 : 6-tri-*O*-methyl-D-fructose, and 4 : 6-di-*O*-methylfructuronic acid (0·5 g.) was obtained as needles, m. p. 107—109°, $[\alpha]_D$ +18·4° (*c*, 0·9 in H_2O).

1 : 3 : 4-*Tri*-O-*methyl*-D-*fructose*.—Methyl 1-*O*-toluene-*p*-sulphonyl-D-fructofuranoside (*C*) (30 g.) in pyridine (450 c.c.) mixed with triphenylmethyl chloride (60 g.) was kept at room temperature for 4 days. After removal of crystalline triphenylmethanol the mixture was poured into ice–water (750 c.c.) with vigorous stirring. *Methyl* 1-O-*toluene*-p-*sulphonyl*-6-O-*trytyl*-D-*fructoside* separated as a sticky gum which hardened to an amorphous solid when washed continuously with water for 2 weeks. An ethereal solution of the solid was washed with dilute acetic acid (pH 4) (4 times), saturated sodium hydrogen carbonate solution (3 times), and water (4 times), then dried (Na_2SO_4), and the solvent removed, giving an amorphous solid *product* (*K*) (55·2 g., 108%), $[\alpha]_D^{18}$ +7·5° (*c*, 2·40 in MeOH) (Found : S, 4·2. $C_{33}H_{34}O_8S$ requires S, 5·8%).

The amorphous solid (*K*) (28·8 g.) in methanol (260 c.c.) and water (50 c.c.) was treated with sodium amalgam (4%) for 60 hr. at 45° and the solid product (19·1 g., 90%), $[\alpha]_D^{18}$ +12° (*c*, 1·9 in MeOH), isolated as before. Methylation thrice with methyl iodide and silver oxide gave a viscous syrup, n_D^{18} 1·5695 (17·5 g. from 18·9 g., 95%), $[\alpha]_D^{19}$ +9·2° (*c*, 1·1 in MeOH) (Found : OMe, 16·7; trityl, 57·6. Calc. for $C_{29}H_{34}O_6$: OMe, 25·9; trityl, 50·8. Calc. for $C_{29}H_{34}O_6$,CPh_3·OH : OMe, 16·8; trityl, 65·8%). All attempts to remove contaminating triphenylmethanol were unsuccessful.

A cooled saturated solution of hydrobromic acid in glacial acetic acid (10 c.c.) was added to crude methyl 1 : 3 : 4-tri-*O*-methyl-6-*O*-trityl-D-fructoside (16 g.) dissolved in glacial acetic acid (30 c.c.) cooled to 0°. After 2 min. the mixture was poured into 0·1N-sulphuric acid (100 c.c.), and the product filtered. The filtrate was heated at 95° for 1 hr. to ensure complete removal of the glycosidic methoxyl group. Extraction with chloroform thrice, and removal of the chloroform, gave 1 : 3 : 4-tri-*O*-methylfructose (4·2 g., 57%) which partly crystallised. The crystals (1·5 g.) after recrystallisation from carbon tetrachloride–light petroleum (b. p. 40—60°) had m. p. 75°, $[\alpha]_D^{20}$ −56·2° (*c*, 1·05 in H_2O) (Found : C, 48·65; H, 8·2; OMe, 41·7. Calc. for $C_9H_{18}O_6$: C, 49·1; H, 8·35; OMe, 41·9%). Chromatographic analysis of the mother liquors (2·74 g.) revealed the presence of tetra-*O*-methylfructose contaminating the 1 : 3 : 4-tri-*O*-methylfructose. Separation was effected on silica (Bell and Palmer, *loc. cit.*). The trimethyl sugar was isolated as a pale yellow syrup (1·31 g.) which slowly crystallised; the solid had m. p. 75°.

1 : 4 : 5-*Tri*-O-*methyl*-D-*fructose*.—2 : 3-4 : 5-Di-*O*-*iso*propylidene-D-fructose (36 g.) dissolved in N-sulphuric acid (1000 c.c.) was kept at 25° for 10 hr. and the solution then neutralised by

passage through anion-exchange resin (IR-4B). The eluate (reduced to 250 c.c. by evaporation) was then extracted thrice with chloroform (500 c.c.); evaporation of the aqueous solution gave a syrup which on chromatographic analysis showed only the presence of 2 : 3-*O-iso*propylidene-fructose and fructose. Attempted separation of these two sugars with boiling acetone–ethyl acetate was reasonably successful, and after removal of the acetone–ethyl acetate the residual syrup was distilled twice at 200°/0·04 mm. The crude 2 : 3-*O-iso*propylidene-D-fructopyranose (7 g.) was acetylated with acetic anhydride and anhydrous sodium acetate; 1 : 4 : 5-tri-*O*-acetyl-2 : 3-*O-iso*propylidene-D-fructose formed prisms (7·38 g.), m. p. 55—56° (from aqueous ethanol), $[\alpha]_D^{18}$ +18° (*c*, 1·1 in ethanol) (Found: C, 51·8; H, 6·35. Calc. for $C_{15}H_{22}O_9$: C, 52·0; H, 6·4%). The acetyl groups were removed with cold N-sodium hydroxide, and syrupy, chromatographically pure, 2 : 3-*O-iso*propylidene-D-fructopyranose (5·1 g.), $[\alpha]_D^{19}$ +29° (*c*, 1·1 in EtOH), was obtained (Wolfrom, Shilling, and Binkley, *J. Amer. Chem. Soc.*, 1950, **72**, 4544, record $[\alpha]_D^{24}$ +28·2° in EtOH, for this compound).

This syrup was methylated five times with methyl iodide and silver oxide and gave after distillation a chromatographically pure syrup (4·51 g., 74%), b. p. 100°/0·09 mm., n_D^{17} 1·4512, $[\alpha]_D^{17}$ +35° (*c*, 1·34 in EtOH). Hydrolysis with sulphuric acid (100 c.c.; 0·1N) at 95° for 2 hr. gave a syrupy *ether* (3·07 g. from 4·4 g., 82%), n_D^{17} 1·4772, $[\alpha]_D^{18}$ −143° (*c*, 1·0 in H_2O) (Found: OMe, 41·0. $C_9H_{18}O_6$ requires OMe, 41·9%), R_G 0·76 and 0·61 solvents I and II as eluant, respectively. The crystalline phenylosazone had m. p. 66—67°, alone or on admixture with the phenylosazone obtained from 4 : 5-dimethylfructose.

3 : 4-*Di*-O-*methyl*-D-*fructose*.—(*a*) Methyl 1-*O*-toluene-*p*-sulphonyl-6-*O*-trityl-D-fructoside (*K*) (25 g.) was methylated with methyl iodide and silver oxide thrice and gave a viscous syrupy *ether* (23·7 g., 91%), n_D^{18} 1·5608, $[\alpha]_D$ +14° (*c*, 1·5 in MeOH) (Found: OMe, 14·9. $C_{35}H_{38}O_8S$ requires OMe, 15·1%). The toluene-*p*-sulphonyl group was more resistant to fission with sodium amalgam in methanol than that of methyl 3 : 4 : 6-tri-*O*-methyl-1-*O*-toluene-*p*-sulphonyl-fructoside, and treatment had to be continued for 30 hr. A viscous *product* (14·5 g. from 22·5 g., 86%), $[\alpha]_D$ +24° (*c*, 1·3 in MeOH), was obtained (Found: OMe, 20·1. $C_{28}H_{32}O_6$ requires OMe, 20·0%). The trityl residue was removed as in the previous synthesis, the sugar being left in contact with hydrobromic acid for a longer period (10 min.). The syrup obtained after hydrolysis with sulphuric acid was purified by successive solution in acetone, ethanol, and chloroform, followed by agitation of an ethanolic solution with anion exchange resin (IR-4B) for 2 hr. An amber-coloured syrup was isolated (4·18 g. from 13·7 g.; 68%) which showed, on chromatographic analysis, the presence of tetra- and tri-*O*-methyl- in addition to di-*O*-methyl-fructose. Separation was effected by elution on powdered cellulose with *n*-butanol–light petroleum (b. p. 100—120°) (3 : 7) as eluant. After the complete removal of the tetra- and tri-*O*-methyl fractions (2·3 g.) the solvent proportions were changed to 1 : 1, and 3 : 4-di-*O*-methylfructose (1·22 g.), n_D^{18} 1·4809, $[\alpha]_D^{14}$ −62° (constant value) (*c*, 3·6 in H_2O), was obtained (Found: OMe, 28·0. Calc. for $C_8H_{16}O_6$: OMe, 29·8%), R_G 0·66 and 0·22 in solvents (I) and (II), respectively.

(*b*) 2 : 3-*O-iso*Propylidene-1 : 6-di-*O*-toluene-*p*-sulphonyl-D-fructose (*L*) (33·9 g., 23%) was prepared from D-fructose (50 g.) by Morgan and Reichstein's method (*loc. cit.*), the yield being improved by the addition of acetaldehyde (0·1 c.c.) during the condensation with acetone; the compound had m. p. 132—133°, $[\alpha]_D^{16}$ +15·0° (*c*, 1·3 in EtOH) (Found: C, 52·5; H, 5·3; S, 11·7. Calc. for $C_{23}H_{26}O_{10}S_2$: C, 52·3; H, 5·3; S, 11·8%). The *iso*propylidene group was removed and the glycoside formed by treatment with 1% methanolic hydrogen chloride; *methyl* 1 : 6-*di*-O-*toluene*-p-*sulphonyl*-D-*fructoside* (*M*) was isolated as a non-reducing glass in 85% yield; it had $[\alpha]_D$ +14·7° (*c*, 1·36 in MeOH) (Found: S, 13·3; OMe, 6·1. $C_{21}H_{26}O_{10}S_2$ requires S, 13·6; OMe, 6·6%). The product (*M*) (13·3 g.) was methylated twice with methyl iodide and silver oxide, and *methyl* 3 : 4-*di*-O-*methyl*-1 : 6-*di*-O-*toluene*-p-*sulphonyl*-D-*fructoside* (*N*) isolated as a viscous syrup (12·5 g., 90%), $[\alpha]_D^{20}$ +20° (*c*, 1·0 in MeOH) (Found: S, 12·0; OMe, 18·7. $C_{23}H_{30}O_{10}S_2$ requires S, 12·1; OMe, 17·6%). Treatment of (*N*) (12·1 g.) with sodium amalgam as above gave a syrup (3·9 g., 77%) which on chromatographic analysis (acid naphtharesorcinol spray) showed slight contamination with methyl tri-*O*-methylfructoside. Attempted purification by fractional distillation was unsuccessful; the main fraction (2·10 g.) distilled at 150°/0·06 mm. as a pale syrup, $[\alpha]_D^{18}$ +31·6° (*c*, 1·1 in MeOH) (Found: OMe, 43·8. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). This (1·8 g.) was hydrolysed to 3 : 4-di-*O*-methylfructose (1·1 g.) with 0·1N-sulphuric acid and purified from tri-*O*-methylfructoses by separation on powdered cellulose as in the previous synthesis of this derivative. A viscous syrup (1·04 g.) was obtained which had n_D^{18} 1·4817, $[\alpha]_D^{16}$ −19·6° (initial), −35° (17 hr.), −39° (40 hr., const.) (*c*, 1·1 in MeOH), −63° (constant value; *c*, 1·1 in H_2O) (Found: OMe, 28·6. Calc. for $C_8H_{16}O_6$: OMe, 29·8%).

Characterisation of 3 : 4-*Di*-O-*methyl*-D-*fructose*.—3 : 4-Di-*O*-methyl-D-fructose (0·74 g.) was oxidised with sodium metaperiodate (40 c.c.) (0·6M) and then with bromine water according to the conditions described by Arni and Percival (*loc. cit.*). Conversion into the methyl ester gave a syrup (0·25 g.) which distilled at 140°/0·03 mm.; the distillate had n_D^{20} 1·4441, $[\alpha]_D^{20}$ −55·1° (*c*, 2·1 in MeOH).

The distilled (−)-dimethoxysuccinate (0·075 g.) on treatment with methanolic ammonia gave (−)-dimethoxysuccinamide (0·05 g.), m. p. 275—276°, $[\alpha]_D^{21}$ −90·3° (*c*, 0·6 in H_2O). The corresponding *NN'*-dimethyl-(−)-dimethoxysuccinamide was obtained in good yield; it had m. p. 204—205°, unchanged on admixture with an authentic specimen, $[\alpha]_D^{20}$ −132° (*c*, 0·85 in H_2O) (Found: C, 47·1; H, 8·1; N, 13·3. Calc. for $C_8H_{16}O_4N_2$: C, 47·0; H, 7·9; N, 13·7%).

4 : 5-*Di*-O-*methyl*-D-*fructose*.—1 : 2-4 : 5-Di-*O*-*iso*propylidene-3-*O*-toluene-*p*-sulphonyl-D-fructose (*F*) (15·9 g.), dissolved in acetic acid (80%; 80 c.c.), was kept at 60° for 3·5 hr. (Ohle and Just, *loc. cit.*). The acetic acid was removed by distillation and the residue dissolved in ether; the ethereal solution was washed with sodium hydrogen carbonate solution (thrice) and with water, and light petroleum (b. p. 60—80°) added to turbidity. Long needles were deposited of 1 : 2-*O*-*iso*propylidene-3-*O*-toluene-*p*-sulphonyl-D-fructose (13 g., 91%), m. p. 124—125°; $[\alpha]_D^{20}$ −112° (*c*, 1·2 in $CHCl_3$), −128° (*c*, 1·2 in MeOH) (Found: C, 51·1; H, 6·1; S, 8·4. Calc. for $C_{16}H_{22}O_8S$: C, 51·3; H, 5·9; S, 8·6%). Methylation thrice with methyl iodide and silver oxide gave a *dimethyl ether* (92%), prisms (from aqueous methanol), m. p. 84—85°, $[\alpha]_D^{17}$ −121° (*c*, 1·0 in MeOH) (Found: C, 53·9; H, 6·4; OMe, 16·5; S, 7·8. $C_{18}H_{26}O_8S$ requires C, 53·7; H, 6·5; OMe, 15·5; S, 8·0%).

The toluene-*p*-sulphonyl group was removed by treatment with 4% sodium amalgam for 48 hr. at 45°. The product, 4 : 5-*di*-O-*methyl*-1 : 2-*O*-iso*propylidene*-D-*fructose*, after distillation at 130°/0·10 mm. and recrystallisation from light petroleum (b. p. 60—80°) formed hygroscopic needles (4·34 g., 94%), m. p. 64—65°, $[\alpha]_D^{18}$ −169° (*c*, 1·0 in MeOH) (Found: C, 53·2; H, 7·9; OMe, 24·0. $C_{11}H_{20}O_6$ requires C, 53·2; H, 8·1; OMe, 25·0%).

4 : 5-Di-*O*-methyl-1 : 2-*O*-*iso*propylidene-D-fructose (4·0 g.) dissolved in sulphuric acid (0·1N; 100 c.c.) was heated at 95° for 3 hr. Neutralisation by passage through anion-exchange resin (IR-4B) and evaporation gave a syrup (3·27 g., 95%) which crystallised completely. The crystals were dissolved in hot ethanol (2 c.c.) and warm carbon tetrachloride (50 c.c.) was added; needles of 4 : 5-*di*-O-*methyl*-D-*fructose* were deposited, m. p. 104—105°, $[\alpha]_D^{15}$ −167° (*c*, 1·3 in H_2O) (Found: C, 46·2; H, 7·9; OMe, 28·3. $C_8H_{16}O_6$ requires C, 46·15; H, 7·7; OMe, 29·8%). These gave on chromatographic analysis a single spot, R_G 0·49 in solvent (I), 0·14 in solvent (II). The crystalline phenylosazone had m. p. 67—68° (from aqueous ethanol), $[\alpha]_D^{19}$ −27·5° ⟶ −8·2°) (*c*, 1·1 in ethanol). Orange needles of 4 : 5-*dimethylfructose* 2 : 5-*dichlorophenylhydrazone*, m. p. 102°, $[\alpha]_D^{17}$ −45° (*c*, 0·2 in H_2O), were obtained on refluxing an alcoholic solution of this dimethyl ether with 2 : 5-dichlorophenylhydrazine (Found: C, 45·0; H, 5·3; N, 7·85. $C_{14}H_{20}O_5N_2Cl_2$ requires C, 45·8; H, 5·4; N, 7·6%).

4-O-*Methyl*-D-*fructose*.—Three methylations with Purdie reagents of 2 : 3-*O*-*iso*propylidene-1 : 6-di-*O*-toluene-*p*-sulphonyl-D-fructose (*L*) (11·4 g.) gave crystalline 4-O-*methyl*-2 : 3-O-iso*propylidene*-1 : 6-*di*-O-*toluene*-p-*sulphonyl*-D-*fructose* (11·6 g., 91%), m. p. 112—113°, $[\alpha]_D^{19}$ +23° (*c*, 1·5 in EtOH) (Found: C, 52·8; H, 5·4; OMe, 5·95. $C_{24}H_{30}O_{10}S_2$ requires C, 53·1; H, 5·6; OMe, 5·7%). Removal of the toluenesulphonyl groups and distillation gave a 4-O-*methyl*-2 : 3-O-iso*propylidene*-D-*fructose* (4·2 g.), b. p. 150°/0·05 mm., $[\alpha]_D$ +6·5° (*c*, 1·5 in EtOH) (Found: OMe, 13·0. $C_{10}H_{18}O_6$ requires OMe, 13·25%).

Hydrolysis of this syrup (3·48 g.) with sulphuric acid (0·1N; 150 c.c.) at 95° for 3 hr. gave 4-*O*-methylfructose (2·91 g., 100%), n_D^{18} 1·4905, $[\alpha]_D^{18}$ −43° ⟶ −61° (72 hr., const.) (*c*, 1·3 in MeOH), −93° ⟶ −97° (4 days, const.) (*c*, 1·0 in H_2O) (McDonald and Jackson, *loc. cit.*, record $[\alpha]_D$ −87·5 in H_2O) (Found: OMe, 16·3. Calc. for $C_7H_{14}O_6$: OMe, 16·0%). Chromatographic analysis of this syrup showed a single spot, R_G 0·37, in solvent (I). The phenylosazone had m. p. 157—158° (from aqueous acetone), undepressed on admixture with authentic 4-*O*-methylglucosazone, $[\alpha]_D^{17}$ −35° ⟶ −14° (*c*, 0·3 in H_2O) (cf. Knauf, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1941, **63**, 1447, who record m. p. 158—159°, $[\alpha]_D$ −36·0 ⟶ −14·4° for 4-*O*-methylglucosazone).

Characterisation of 4-O-*Methyl*-D-*fructose*.—Oxidation of the above syrup (0·50 g.) was carried out as for the 3 : 4-di-*O*-methylfructose. The dimethyl (−)-hydroxymonomethoxysuccinate (0·128 g.) had n_D^{17} 1·4510, $[\alpha]_D^{17}$ −41° (*c*, 1·3 in MeOH). Crystalline D-*threo*-2-hydroxy-3-methoxysuccinamide had m. p. 198—200°. The *bismethylamide* was also obtained and after recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave long needles, m. p.

137°, $[\alpha]_D$ −103° (*c*, 0·6 in H_2O) (Found: C, 44·2; H, 7·4; N, 13·5. $C_7H_{14}O_4N_2$ requires C, 44·2; H, 7·4; N, 14·7%). These two derivatives have been synthesised from tartaric acid and had melting points and rotation identical with the above.

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The Periodate Oxidation of Methylfructoses.

By W. E. A. Mitchell and Elizabeth Percival.

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The extent of periodate oxidation of a number of methyl ethers of fructose has been measured by the determination of liberated formaldehyde and also by periodate uptake.

Periodate oxidation of methylated monosaccharides has been studied extensively by Bell (*J.*, 1948, 992), and Bell, Palmer, and Johns (*J.*, 1949, 1536). In the latter paper, the oxidation of 1 : 3 : 4- and 3 : 4 : 6-tri-*O*-methyl- and 3 : 4-di-*O*-methyl-D-fructose was described, and the liberated formaldehyde determined as its dimedone derivative. These workers found that a quantitative yield of formaldehyde was frequently difficult to obtain. Experiments on the molar uptake of periodate carried out concurrently gave much higher results than would be expected. This phenomenon was also observed by Hirst and Jones (*J.*, 1949, 1659) and Greville and Northcote (*J.*, 1952, 1945) during the oxidation of certain methylaldoses, and also by Neumüller and Vasseur (*Arkiv. för Kemi*, 1953, 235), while studying the action of periodate on disaccharides at different pH's.

In the present work the methyl ethers of fructose were oxidised at room temperature in the dark, and in view of the earlier anomalous results the formaldehyde obtained was determined both gravimetrically, by Bell's method (*loc. cit.*), and colorimetrically with chromotropic acid (see Eegriwe, *Z. analyt. Chem.*, 1937, **110**, 22); the quantity of periodate consumed was also measured. In order to determine the formaldehyde by the colorimetric method it is necessary first to destroy the excess of periodate, *e.g.* with stannous chloride (Corcoran and Page, *J. Biol. Chem.*, 1940, **146**, 279) or with sodium arsenite (Lambert and Neish, *Canad. J. Res., B.*, 1950, **28**, 83). The procedure finally adopted by the present authors was a modification of a method communicated by Mr. M. W. Rees of Cambridge (unpublished work), to whom they would like to record their thanks. The excess of periodate was removed by sodium hydrogen sulphite solution, and with each set of determinations a standard curve was constructed for glucose, the oxidation of which with liberation of formaldehyde takes place in a few minutes. This curve was linear up to a concentration of approximately 2 μg./ml. of solution. Although difficulties have been reported in the use of chromotropic acid we found good agreement between this method and the gravimetric method in experiments carried out on the same sample. The principal advantages of the colorimetric method are the speed of determination when several sugars are examined and the small amount of sugar, 200—500 μg., required for each estimation.

Although the yields of formaldehyde obtained from the methylated fructoses were reasonably high, yields of 100% were rarely obtained. The exceptions were occasional oxidations in bicarbonate buffer, but results in this medium were variable. The results for 3 : 4 : 6-tri-*O*-methyl- and for 3 : 4-di-*O*-methyl-D-fructose were in agreement with those recorded by Bell *et al.* (*loc. cit.*). The yield of formaldehyde from 1 : 3 : 4-tri-*O*-methylfructose by the colorimetric method was low and did not agree with the theoretical yield recorded by the above authors.

Fructose, 4-*O*-methyl-, 3 : 4-di-*O*-methyl-, and 3 : 4 : 6-tri-*O*-methyl-D-fructose gave nearly theoretical uptakes of periodate, but in every other derivative examined over-oxidation was encountered. With 1 : 4 : 5- and 1 : 4 : 6-tri-*O*-methylfructose the uptake of periodate after 24 hr. was extremely slow and did not exceed 1·4 mol. of periodate with either sugar. There does not appear to be any analogy with the results obtained by Northcote and Greville (*loc. cit.*) from similar methylated glucoses. The latter authors found for 2 : 4 : 6-tri-*O*-methylglucose a periodate consumption of 4·13 mol. of periodate during 294 hr. We consider that, under the mildly alkaline conditions of the experiment,

partial enolisation may occur in these two methylated fructoses between $C_{(2)}$ and $C_{(3)}$, and that this is followed by attack by periodate.

TABLE 1. *Determination of formaldehyde gravimetrically.*

Sugar	Buffer	Duration of oxidn., hr.	H·CHO liberated, mol.	H·CHO expected, mol.
Fructose	Phosphate	48	1·70	2·0
	Bicarbonate	48	1·68	—
3 : 4 : 6-Tri-*O*-methylfructose	Phosphate	48	0·88	1·0
	,,	72	0·89	—
	No buffer	72	0·88	—
1 : 4 : 6-Tri-*O*-methylfructose	,,	72	0·10 *	0·0
	Phosphate	72	0·27	—
4-*O*-Methylfructose	No buffer	72	1·40	2·0
	Phosphate	72	1·38	—

* Impure precipitate—low m. p.

TABLE 2. *Determination of formaldehyde colorimetrically.*

Sugar	Buffer	H·CHO liberated, mol. Green filter		Yellow filter		H·CHO expected, mol.
Fructose	Phosphate	1·6	1·7	—	1·72	2·0
	Bicarbonate	1·9	1·4	—	—	—
4-*O*-Methylfructose	Phosphate	1·66	1·76	—	1·70	2·0
	Bicarbonate	—	1·4	—	—	—
3 : 4-Di-*O*-methylfructose	Phosphate	1·58	1·62	—	1·76	2·0
	Bicarbonate	1·76	1·94	—	—	—
4 : 5-Di-*O*-methylfructose	Phosphate	0·83	0·85	0·83	0·90	1·0
	Bicarbonate	0·73	0·80	—	—	—
1 : 3 : 4-Tri-*O*-methylfructose	Phosphate	0·85	0·81	0·78	0·81	1·0
	Bicarbonate	0·79	0·82	—	—	—
3 : 4 : 6-Tri-*O*-methylfructose	Phosphate	0·92	0·89	0·84	0·92,0·90	1·0
	Bicarbonate	0·81	1·00	—	—	—

TABLE 3. *Molar consumption of periodate.*

Sugar	Duration of oxidn., hr.	Uptake, mol.	Expected uptake, mol.
Fructose	48	5·0	5·0
4-*O*-Methylfructose	48	2·7	3·0
	72	2·8	—
3 : 4-Di-*O*-methylfructose	48	2·0	2·0
4 : 5-Di-*O*-methylfructose	48	2·3	2·0
	72	2·5	—
1 : 3 : 4-Tri-*O*-methylfructose	48	1·6	1·0
	72	1·5	—
3 : 4 : 6-Tri-*O*-methylfructose	48	0·8	1·0
1 : 4 : 6-Tri-*O*-methylfructose	24	1·2	1·0
	48	0·9	—
	72	1·3	—
1 : 4 : 5-Tri-*O*-methylfructose	24	1·2	1·0
	48	1·3	—
	72	1·4	—
	168	1·4	—

EXPERIMENTAL

The buffer solutions (pH 7·5, verified in a pH meter) used in the determinations were molar sodium hydrogen carbonate (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476), and 0·1M-citric acid (1·5 ml.) mixed with 0·2M-disodium hydrogen phosphate (18·5 ml.).

Estimation of Formaldehyde.—(a) *Gravimetric.* Bell's method (*loc. cit.*) was followed, a large excess of periodate being used, together with a crystallisation time of 18 hr.

(b) *Colorimetric.* A solution of the sugar (5 ml.) (sufficient to produce 50—100 μg. of formaldehyde) was mixed with phosphate buffer solution (0·5 ml.; pH 7·5) or with sodium hydrogen carbonate solution (M; 0·1 ml.), and sodium metaperiodate solution (0·3M; 0·1 ml.) in a tared boiling tube, and the stoppered mixture kept in the dark for 48 hr. Sodium hydrogen sulphite solution (*s.g.* 1·34; 0·2 ml.) was added to destroy excess of periodate, and, after shaking, chromotropic acid (4 : 5-dihydroxynaphthalene-2 : 7-disulphonic acid) (0·1M; 0·5 ml.) added. Concentrated sulphuric acid (20 ml.) was added slowly with cooling in a freezing-mixture. The

colour was developed by heating the solution for 10 min. at 85°. After cooling, the volume of the solution was adjusted to 65 ml. Blank experiments were carried out in each determination.

The purple solution was examined in an absorptiometer, green (Ilford 604) and yellow (Ilford 606) filters being used. A calibration curve was constructed for each set of estimations, with glucose as a standard, and straight lines were obtained, with each filter, for quantities of formaldehyde from 10 to 120 μg.

Periodate Uptake.—The amount of periodate consumed was determined by Fleury and Lange's method (*J. Pharm. Chim.*, 1933, **17**, 107, 196). The oxidation was carried out in a bicarbonate buffer (1 g./10 ml. of solution).

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Products from the Alkaline and Reductive Fission of the Epoxide Ring of Methyl 3 : 4- *and* 2 : 3-*Anhydro*-6-*deoxy*-α-L-*taloside and of their Methylated Derivatives.*

By George Charalambous and Elizabeth Percival.

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6-Deoxy-3-*O*-methyl-L-idose and 6-deoxy-4-*O*-methyl-L-mannose have been isolated from the alkaline fission of methyl 3 : 4-anhydro-6-deoxy-α-L-taloside. Methylation of the hydroxyl group at position 2, before fission, led to the isolation of 6-deoxy-2 : 4-di-*O*-methyl-L-mannose. Similar treatment of the 2 : 3-anhydro-derivatives gave rise to 6-deoxy-3-*O*-methyl-L-idose and 6-deoxy-2 : 4-di-*O*-methyl-L-galactose. Reduction of these epoxide ring compounds with lithium aluminium hydride afforded dideoxy-sugars.

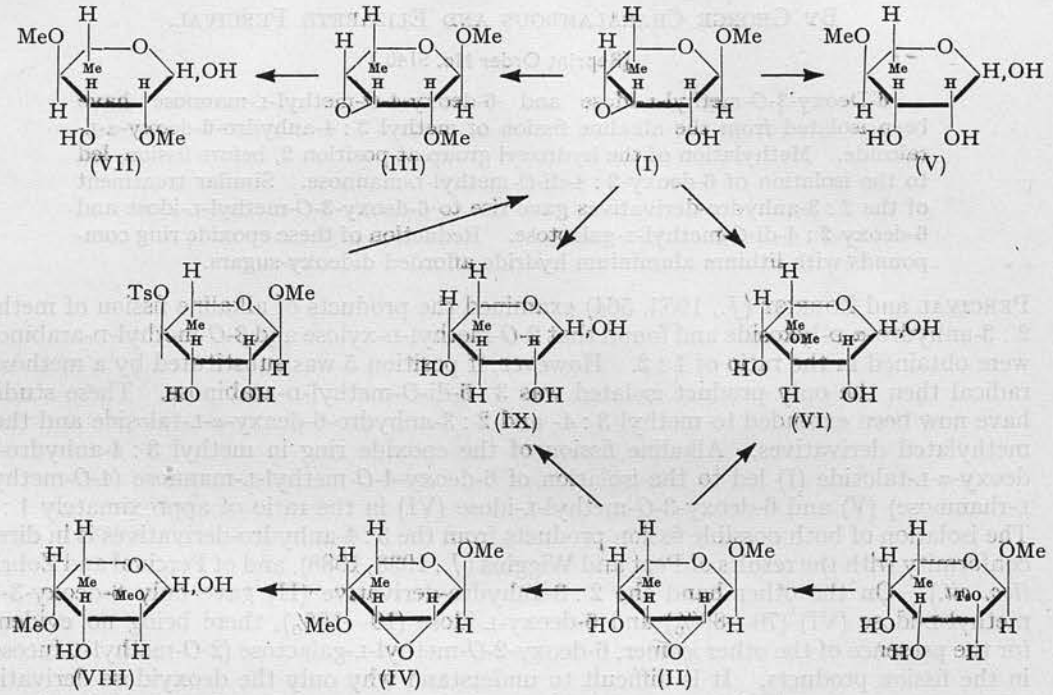
Percival and Zobrist (*J.*, 1953, 564) examined the products of alkaline fission of methyl 2 : 3-anhydro-α-D-lyxoside and found that 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-arabinose were obtained in the ratio of 1 : 2. However, if position 5 was substituted by a methoxyl radical then the only product isolated was 3 : 5-di-*O*-methyl-D-arabinose. These studies have now been extended to methyl 3 : 4- and 2 : 3-anhydro-6-deoxy-α-L-taloside and their methylated derivatives. Alkaline fission of the epoxide ring in methyl 3 : 4-anhydro-6-deoxy-α-L-taloside (I) led to the isolation of 6-deoxy-4-*O*-methyl-L-mannose (4-*O*-methyl-L-rhamnose) (V) and 6-deoxy-3-*O*-methyl-L-idose (VI) in the ratio of approximately 1 : 2. The isolation of both possible fission products from the 3 : 4-anhydro-derivatives is in direct conformity with the results of Peat and Wiggins (*J.*, 1938, 1088), and of Percival and Zobrist (*loc. cit.*). On the other hand the 2 : 3-anhydro-derivative (II) gave only 6-deoxy-3-*O*-methyl-L-idose (VI) (70—80%) and 6-deoxy-L-idose (10—15%), there being no evidence for the presence of the other isomer, 6-deoxy-2-*O*-methyl-L-galactose (2-*O*-methyl-L-fucose), in the fission products. It is difficult to understand why only the deoxyidose derivative could be found, but steric factors may hinder the formation of the galactose derivative, as Gyr and Reichstein (*Helv. Chim. Acta*, 1945, **28**, 226) record the isolation of only the 3-*O*-methyl-D-idose derivative from methyl 2 : 3-anhydro-4 : 6-*O*-benzylidene-α-D-taloside, and Sorkin and Reichstein (*ibid.*, p. 1) the 2-*O*-methyl-D-idose derivative from methyl 2 : 3-anhydro-4 : 6-*O*-benzylidene-α-D-guloside.

6-Deoxy-3-*O*-methyl-L-idose has not been described previously and two identical fractions (1*a* and 1*b*), obtained respectively from the sodium methoxide fission of the epoxide ring of the 3 : 4- and the 2 : 3-anhydro-derivatives of talose, may presumably have this constitution as it is the only possible mono-*O*-methyl derivative which can be obtained by this means from both derivatives. Fischer, Bollinger, and Reichstein (*ibid.*, 1954, **37**, 6) have reported, since this work was completed, the synthesis of 6-deoxy-3-*O*-methyl-D-idose and its osazone. The constants recorded for these derivatives are the same (rotations of opposite sign) as those reported by us for the corresponding derivatives of the L-sugar.

From the methylated 3 : 4-anhydro- (III) and 2 : 3-anhydro- (IV) derivatives of talose only a single product was isolated : 6-deoxy-2 : 4-di-*O*-methyl-L-mannose (VII) from the former and 6-deoxy-2 : 4-di-*O*-methyl-L-galactose (VIII) from the latter. Butler, Lloyd, and Stacey (*Chem. and Ind.*, 1954, 107) have reported the synthesis of 6-deoxy-2 : 4-di-*O*-methyl-L-mannose by a different route, and our proof of the constitution of (VII) is based partly on the aniline derivative which is identical with the 6-deoxy-2 : 4-di-*O*-methyl-*N*-phenylmannosylamine synthesised by these authors, and partly on the fact that no evidence could be obtained for the presence of the other possible isomer, 6-deoxy-2 : 3-di-*O*-methyl-

L-idose. Complete methylation of (VII) proved difficult and the syrupy trimethyl ether isolated was contaminated with breakdown products as shown by its negative rotation and the poor yield of aniline derivative. However, oxidation of this ether, followed by esterification and amide formation, gave crystalline 2 : 3 : 4-tri-*O*-methyl-L-arabaramide, proving that the syrupy trimethyl ether was in fact mainly 6-deoxy-2 : 3 : 4-tri-*O*-methylmannose.

The isolation of the 6-deoxy-L-idose in greater quantity from the unmethylated 3 : 4-epoxide does not support the statement by Bose, Chaudhuri, and Bhattacharyya (*Chem. and Ind.*, 1953, 869) that " the opening of the 3 : 4-epoxide ring seems to follow the rule that the hydroxyl group at position 4 must be *trans* to the bulky primary hydroxyl group " while the isolation of 6-deoxy-2 : 4-di-*O*-methylmannose (VII) from the 2-methyl-3 : 4-



epoxide (III) is in agreement with these authors' contention. It may well be, however, that the methyl group, which in the present experiments has replaced the primary hydroxyl group, has less influence on the scission of the epoxide ring. At the same time these authors, in an extension of Fürst and Plattner's rule (12*th Int. Cong. Pure and Appl. Chem.*, 1951, *Abs.*, p. 409), express the view that the predominant product from the scission of the epoxide ring in 2 : 3-anhydro-sugars has the entering group in the axial position, and the isolation of 6-deoxy-3-*O*-methyl-L-idose (VI) from methyl 2 : 3-anhydro-6-deoxy-α-L-taloside (II) is in direct support of this view. This is not true however for the product (VIII) from the fission of methyl 2 : 3-anhydro-6-deoxy-4-*O*-methyl-α-L-taloside (IV), the entering group being equatorial (C1 conformation). It appears that the presence of a methoxyl group in the molecule has a definite directing effect in the scission of the epoxide ring.

A syrup with similar rotations and the same R_G value was obtained from the action of lithium aluminium hydride on the methyl 3 : 4- and 2 : 3-anhydro-6-deoxy-α-L-talosides, (I) and (II). Consideration of the formulæ of these two substances makes it clear that the product must be 3 : 6-dideoxytalose (3 : 6-dideoxyidose) (IX). As the reduction product from the 3 : 4-anhydro-2-*O*-methyl derivative (III) on demethylation gave a syrup with the same R_G as (IX) it may be tentatively assumed that this product is the 3 : 6-dideoxy-2-*O*-methyltalose. No conclusions can be drawn, from the evidence obtained, of the constitution of the main product from the action of lithium aluminium hydride on the 2 : 3-anhydro-4-*O*-methyl derivative (IV).

Experimental

All solvents were removed under reduced pressure and below 50°. The R_G values were determined with *n*-butanol–ethanol–water (4 : 1 : 5).

Methyl 3-: 4-Anhydro-6-deoxy-α-L-taloside (I).—An ethanolic (50 ml.) solution of methyl 4-*O*-toluene-*p*-sulphonyl-α-L-rhamnoside (20·0 g., from 25 g. of rhamnose hydrate), prepared according to the method described by Percival and Percival (*J.*, 1950, 690), was titrated with sodium hydroxide (2M; 30·0 c.c.) at 75° until it was permanently pink to phenolphthalein. Sodium toluenesulphonate was filtered off, and the solution evaporated to dryness. Extraction of the residue with dry ethyl acetate (5 × 20 c.c.) at room temperature, removal of the solvent, and recrystallisation of the residue from warm light petroleum (b. p. 40—60°) gave crystalline *methyl 3-: 4-anhydro-6-deoxy-α-L-taloside* (I) (8·0 g.), m. p. 68°, $[\alpha]_D^{15}$ −110° (*c*, 1·1 in H_2O) (Found: C, 52·7; H, 7·4. $C_7H_{12}O_4$ requires C, 52·5; H, 7·5%).

Alkaline Hydrolysis of Methyl 3-: 4-Anhydro-6-deoxy-α-L-taloside.—(a) *With barium hydroxide.* Crystalline (I) (0·05 g.) was heated with barium hydroxide (1 g.) in water (5 c.c.) at 100° for 2 hr. After suitable treatment a syrup (0·025 g.), $[\alpha]_D^{14}$ −60° (*c*, 0·4 in H_2O), was obtained (cf. methyl 6-deoxy-α-L-mannoside, $[\alpha]_D^{16}$ −62° in H_2O). Hydrolysis with N-sulphuric acid gave a reducing syrup which partly crystallised when kept. The crystals had m. p. 91° alone and admixed with 6-deoxy-L-mannose. Examination of the mother liquors on a paper chromatogram showed two discrete spots, R_G 0·40 and 0·50, identical with those of 6-deoxy-L-mannose and 6-deoxy-L-idose which were run as controls. The 6-deoxy-L-mannose was present in much the larger quantity.

(b) *With sodium methoxide.* (I) (2·5 g.) was heated at 80° for 19 hr. with dry methanol (150 c.c.) containing sodium methoxide [from sodium (2·0 g.)]. The solution was neutralised with solid carbon dioxide and evaporated to dryness. Extraction with chloroform (6 × 50 c.c.) and removal of the solvent gave a mobile syrup (2·0 g.), b. p. 120—130°/0·1 mm., n_D^{12} 1·4685, $[\alpha]_D^{16}$ −117° (*c*, 2·0 in H_2O). This (1·2 g.) was hydrolysed at 100° with N-sulphuric acid (52 c.c.) until the rotation became constant ($[\alpha]_D^{15}$ −40°; 4 hr.). Neutralisation of the solution with barium carbonate and evaporation to dryness gave a syrup (1·02 g.). Examination on a paper chromatogram showed two discrete spots, R_G 0·75 and 0·66, together with faint spots which corresponded to 6-deoxyidose, R_G 0·50, and 6-deoxymannose, R_G 0·40. The mixture was separated by passage through cellulose (Chanda, Hirst, and Percival, *J.*, 1951, 1240). Elution was by purified light petroleum (b. p. 100—120°)–*n*-butanol (1 : 1; v/v) saturated with water. Fraction 1*a* was a syrup (0·575 g., 56·9%), R_G 0·75, M_G 0·80 (Consden and Stanier, *Nature*, 1952, **170**, 1069; Foster and Stacey, *J. Appl. Chem.*, 1953, **3**, 19), $[\alpha]_D^{16}$ −14° (*c*, 0·7 in H_2O), −13° (*c*, 1·0 in EtOH), n_D^{18} 1·4790 (Found: C, 47·8; H, 8·6; OMe, 18·0. Calc. for $C_7H_{14}O_5$: C, 47·1; H, 7·9; OMe, 17·4%). In one experiment, crystals (0·045 g.) were obtained; they had m. p. 113—114°, R_G 0·75, M_G 0·80, $[\alpha]_D^{17}$ +14·4° (*c*, 2·5 in EtOH), −15° (constant) (*c*, 1·0 in H_2O) (Found: C, 46·8; H, 7·95; OMe, 17·2%). Appropriate treatment of fraction 1*a* (0·065 g.) gave *6-deoxy-3-O-methyl-N-phenyl-L-idosylamine*, m. p. 62—63° (Found: C, 61·0; H, 8·0; N, 4·5. $C_{13}H_{19}O_4N$ requires C, 61·4; H, 7·9; N, 5·4%). Fraction 1*a* (30 mg.) in water (2 c.c.), phenylhydrazine (0·15 c.c.), and glacial acetic acid (2 drops) was heated at 100° for 2 hr. in an atmosphere of carbon dioxide. The solid obtained on cooling was recrystallised from aqueous ethanol, giving pale yellow needles of *6-deoxy-3-O-methyl-L-idosazone*, m. p. 122—123°, $[\alpha]_D^{16}$ −60° (*c*, 1·6 in EtOH) (Found: OMe, 8·4; N, 14·9. $C_{19}H_{24}O_3N_4$ requires OMe, 8·7; N, 15·8%). Fraction 2*a* was a syrup (0·270 g., 26·8%), R_G 0·66, which crystallised completely when kept; the solid had R_G 0·65, M_G 0·58, m. p. 122°, $[\alpha]_D^{15}$ +15° (*c*, 1·0 in MeOH) (Found: C, 47·2; H, 8·1; OMe, 17·2. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 17·4%). Levene and Muskat (*J. Biol. Chem.*, 1934, **105**, 431) record m. p. 122° and $[\alpha]_D$ +13° (in MeOH) for 6-deoxy-4-*O*-methylmannose. Fraction 3*a*, a syrup (0·051 g., 5·05%), had R_G 0·50, $[\alpha]_D^{16}$ −27·4° (*c*, 1·5 in H_2O). Meyer and Reichstein (*Helv. Chim. Acta*, 1946, **29**, 149) record $[\alpha]_D^{15}$ −26° (*c*, 4·462 in H_2O) for 6-deoxy-L-idose. Fraction 4*a* (0·060 g., 5·9%), R_G 0·40, crystallised completely and then had m. p. 92—94°, alone and mixed with authentic 6-deoxymannose.

Methyl 3-: 4-Anhydro-6-deoxy-2-O-methyl-α-L-taloside (III).—Crystalline methyl 3-: 4-anhydro-6-deoxy-α-L-taloside (I) (3·0 g.) was methylated four times with methyl iodide and silver oxide, and a mobile syrup (2·8 g.) was isolated, which distilled at 80—100°/0·01 mm. and had n_D^{15} 1·4500; $[\alpha]_D^{15}$ −140° (*c*, 0·6 in MeOH) (Found: OMe, 35·8. $C_8H_{14}O_4$ requires OMe, 35·6%).

Alkaline hydrolysis with sodium methoxide. Following the procedure used for the unmethylated material the syrup (III) (3·80 g.) was treated with sodium methoxide solution (250 c.c.). A mobile syrup (3·73 g.), $[\alpha]_D^{16}$ −60° (*c*, 1·0 in H_2O), was obtained. This was hydrolysed with N-sulphuric acid to constant rotation ($[\alpha]_D^{16}$ −12°; 3 hr.), and a syrup (*A*) (3·2 g.) which partly

crystallised on storage at 0° was obtained. The crystals (VII) (1·17 g.) had m. p. 82°, $[\alpha]_D^{16}$ −19° (*c*, 1·0 in H_2O), +14·5° ⟶ −3° (48 hr. constant) (*c*, 1·8 in EtOH) (Found : C, 49·5; H, 8·1; OMe, 31·7. Calc. for $C_8H_{16}O_5$: C, 49·9; H, 8·4; OMe, 32·3%). The mother liquors (2·03 g.) from (*A*) on chromatographic analysis showed a main spot, R_G 0·86, and a fainter spot, R_G 1·0. Purification, by passage through powdered cellulose as described previously, gave a further yield of crystals (VII) (0·8 g.), m. p. 82°, a residual syrup (*B*), R_G 0·86, (0·81 g.), and a small quantity of a second fraction, R_G 1·0, $[\alpha]_D$ +20° (*c*, 0·05 in H_2O).

Characterisation of 6-*Deoxy*-2 : 4-*di*-O-*methylmannose*.—The crystals (VII) (0·05 g.) were demethylated by hydriodic acid (2 c.c.) at 100° for 10 min. A syrup which on chromatographic analysis gave a single spot identical with that given by 6-deoxymannose was isolated. Demethylation of syrup (*B*) which was chromatographically and ionophoretically identical with the crystals (VII) gave rise to a similar syrup which revealed only the presence of 6-deoxymannose.

The crystals (VII) (100 mg.) were oxidised with bromine water (1 c.c.) at 15° for 96 hr. The lactone (75 mg.), isolated in the usual way, had $[\alpha]_D^{15}$ +47° (*c*, 0·9 in H_2O) unchanged after 70 hr.; it failed to crystallise or to yield a crystalline amide or phenylhydrazide.

The crystals (VII) (0·1 g.) were heated at 80° for 6 hr. with ethanol (6 c.c.) containing aniline (0·55 c.c.) in the presence of Drierite (0·2 g.). Removal of the solvent at room temperatures gave 6-deoxy-2 : 4-di-*O*-methyl-*N*-phenylmannosylamine, m. p. 141—142°, $[\alpha]_D^{16}$ +110° ⟶ +7° (20 hr.) (*c*, 0·4 in EtOH) (Found : C, 60·8; H, 7·5; OMe, 23·3; N, 5·4. Calc. for $C_{14}H_{21}O_4N$: C, 62·9; H, 7·9; OMe, 23·2; N, 5·2%). An *X*-ray powder photograph of this aniline derivative obtained through the courtesy of Dr. C. A. Beevers was pronounced identical with one of 6-deoxy-2 : 4-di-*O*-methyl-*N*-phenylmannosylamine synthesised in the Birmingham laboratories and supplied by Professor M. Stacey, F.R.S. (*Chem. and Ind.*, 1954, 107). The residual syrup (*B*) gave an identical aniline derivative, m. p. 141—142° alone and mixed with the derivative prepared from the crystals.

The dimethyl sugar (VII) (0·165 g.) was converted into the glycoside (0·150 g.) with 1%-methanolic hydrogen chloride, and this was followed by four methylations with methyl iodide and silver oxide. A mobile syrup (0·145 g.), n_D^{16} 1·4421, $[\alpha]_D^{16}$ −17° (*c*, 1·5 in H_2O), was isolated. Hirst and Macbeth (*J.*, 1926, 22) record n_D^{15} 1·4423, $[\alpha]_D^{16}$ −15° (in H_2O), for methyl 6-deoxy-2 : 3 : 4-tri-*O*-methyl-L-mannoside (Found : OMe, 55·6. Calc. for $C_{10}H_{20}O_4$: OMe, 56·3%). Hydrolysis with N-sulphuric acid gave a syrup, $[\alpha]_D^{17}$ −12° (*c*, 0·5 in H_2O), −24° (*c*, 0·8 in EtOH) {Hirst and Macbeth, *loc. cit.*, record $[\alpha]_D$ +25° (in H_2O), −9° (in ethanol), for 6-deoxy-2 : 3 : 4-tri-*O*-methyl-L-mannose}, which on chromatographic analysis gave a single discrete spot (R_G 1·01) identical with the spot given by authentic 6-deoxy-2 : 3 : 4-tri-*O*-methyl-L-mannose. Attempted conversion into 6-deoxy-2 : 3 : 4-tri-*O*-methyl-*N*-phenylmannosylamine gave a very poor yield of crystals, m. p. and mixed m. p. 111—112°.

Oxidation of the trimethyl ether (0·30 g.) with nitric acid (5·5 c.c.; *d*, 1·42) (Mullan and Percival, *J.*, 1940, 1505) gave a syrup (0·06 g.) which was esterified with boiling methanolic hydrogen chloride (2%; 3·5 c.c.) for 6 hr. The derived amide, $[\alpha]_D^1$ '+52° (*c*, 0·5 in EtOH), partly crystallised, m. p. and mixed m. p. 230°. Hirst and Macbeth (*loc. cit.*) record $[\alpha]_D^{16}$ +50·4° (in H_2O) for 2 : 3 : 4-tri-*O*-methyl-L-arabaramide.

Methyl 2 : 3-*Anhydro*-6-*deoxy*-α-L-*taloside* (II).—Crystalline methyl 6-deoxy-2-*O*-toluene-*p*-sulphonyl-α-L-galactoside (3·30 g.), prepared from 6-deoxygalactose by Percival and Percival's method (*loc. cit.*), was titrated with sodium hydroxide (2M; 9 c.c.) as described for the corresponding 6-deoxymannose derivative. Removal of ethyl acetate gave an extremely volatile crystalline product (II) (1·70 g.) which after recrystallisation from ethanol had m. p. 95—97°, $[\alpha]_D^{16}$ −88° (*c*, 2·0 in H_2O) (Found : C, 52·45; H, 7·3. $C_7H_{12}O_4$ requires C, 52·5; H, 7·5%). This *taloside* was very soluble in alcohol, acetone, chloroform, and light petroleum.

Alkaline hydrolysis. Hydrolysis of (II) with sodium methoxide as described above gave a syrup (0·32 g. from 0·35 g.). Removal of the glycosidic group with N-sulphuric acid afforded a syrup (*C*) (0·21 g.) which on chromatographic analysis showed two spots, R_G 0·75 and 0·50. Separation on cellulose gave two fractions. Fraction 1*b* was a syrup (0·150 g.), $[\alpha]_D^{16}$ −14° (*c*, 0·7 in H_2O) (Found : OMe, 17·5. Calc. for $C_7H_{14}O_5$: OMe, 17·4%), which was chromatographically and ionophoretically identical with fraction I*a*, obtained by similar treatment of the 3 : 4-anhydro-derivative. 6-Deoxy-3-*O*-methyl-*N*-phenyl-L-idosylamine, m. p. and mixed m. p. 62—63°, and 6-deoxy-3-*O*-methyl-*N*-phenyl-L-idosazone, m. p. and mixed m. p. 122—123°, were also prepared from fraction I*b*. Fraction 2*b*, a syrup (0·030 g.; 15%), $[\alpha]_D^{16}$ −22° (*c*, 1·0 in H_2O), R_G 0·50, was presumed to be 6-deoxy-2-*O*-methylgalactose. Nucleation, however, failed to induce crystallisation, and chromatographic comparison showed that authentic 6-deoxy-2-*O*-

methylgalactose has R_G 0·59. Paper ionophoresis of 6-deoxy-2-*O*-methylgalactose gave M_G 0·33 while fraction 2*b* gave M_G 1·0 and 6-deoxygalactose 0·92. 6-Deoxy-L-idose (fraction 3*a*) had R_G 0·59, M_G 1·0.

Methyl 2 : 3-*Anhydro*-6-*deoxy*-4-O-*methyl*-α-L-*taloside* (IV).—(II) (1·01 g.) was methylated four times with methyl iodide and silver oxide. Recrystallisation of the *taloside* (IV) from acetone–light petroleum (b. p. 40—60°) gave long needles, m. p. 108—110°, $[\alpha]_D^{16}$ ±0° (*c*, 0·7 in EtOH, $CHCl_3$, and $COMe_2$) (Found : C, 54·7; H, 8·1; OMe, 35·0. $C_8H_{14}O_4$ requires C, 55·2; H, 8·1; OMe, 35·6%).

Alkaline hydrolysis. Methyl 2 : 3-anhydro-6-deoxy-4-*O*-methyl-α-L-taloside (IV) (0·90 g.) was hydrolysed with sodium methoxide as described for the 3 : 4-anhydro-derivative. The resultant syrup (0·70 g.) after hydrolysis with sulphuric acid had $[\alpha]_D^{18}$ −15° (*c*, 2·4 in MeOH) and gave, on chromatographic analysis, a single spot R_G 0·80 (Found : OMe, 31·8. $C_8H_{16}O_5$ requires OMe, 32·3%).

Characterisation of the syrupy dimethyl compound, R_G 0·80. Demethylation of a portion (0·050 g.) of the syrup with hydriodic acid (*d*, 1·7; 2 c.c.) gave a product which was chromatographically indistinguishable from 6-deoxy-L-galactose.

The syrup, R_G 0·80 (0·235 g.), was converted into the glucoside by methanolic hydrogen chloride (2%; 24 c.c.) at 80°, until it did not reduce Fehling's solution (3 hr.). The glycoside, $[\alpha]_D^{16}$ −30° (*c*, 1·5 in EtOH), was methylated five times with methyl iodide and silver oxide; the resultant methyl tri-*O*-methylglycoside crystallised spontaneously. The crystals (0·15 g.) had m. p. and mixed m. p. 93—95°, $[\alpha]_D^{16}$ −200° (*c*, 1·0 in H_2O) (Found : C, 54·05; H, 9·1; OMe, 55·4. Calc. for $C_{10}H_{20}O_5$: C, 54·55; H, 9·1; OMe, 56·3%) [James and Smith, *J.*, 1945, 746, record m. p. 85—92°, $[\alpha]_D$ −196° (*c*, 0·5 in H_2O), for methyl 6-deoxy-2 : 3 : 4-tri-*O*-methyl-α-L-galactoside.]

Dideoxy-sugars derived by the Action of Lithium Aluminium Hydride on Methyl Anhydro-6-*deoxytalosides.*—Each of the above anhydro-derivatives (I—IV) was treated with lithium aluminium hydride, and the products examined chromatographically. All attempts to prepare crystalline aniline derivatives, phenylosazones, and toluene-*p*-sulphonyl derivatives failed. A typical reduction was carried out as follows. Crystalline methyl 3 : 4-anhydro-6-deoxy-α-L-taloside (I) (2·0 g.) in dry ether (150 c.c.) was added dropwise during 45 min. to a gently refluxing suspension of finely powdered lithium aluminium hydride (2·0 g.) in dry ether (150 c.c.) under vigorous stirring, and the reaction allowed to continue a further 4 hr. The flask was cooled in ice-water, excess of lithium aluminium hydride destroyed by the careful addition of water, and the mixture made acid (2N-sulphuric acid). Exhaustive extraction with chloroform (10 × 100 c.c.) afforded, after purification with Filter Cel, a syrup (0·7 g.) which was hydrolysed with N-sulphuric acid; the resultant reducing syrup showed a single discrete spot on chromatographic analysis.

Similar treatment of the other anhydro-derivatives (II—IV) gave syrups contaminated with 6-deoxy-idose and -galactose and separation on cellulose was necessary. In addition the methylated anhydro-sugars (III) and (IV) also gave some of the products isolated from (I) and (II). The constants of the main products after separation were :

Product from methyl 6-deoxy-α-L-taloside	Yield, %	$[\alpha]_D^{16}$ in MeOH	$[\alpha]_D^{16}$ in H_2O	OMe, %	R_G
3 : 4-Anhydro-	38	−20°	−16°	—	0·72
2 : 3-Anhydro-	11	−18	−20	—	0·72
3 : 4-Anhydro-2-*O*-methyl-	17	−14	− 4	19·2	0·90
2 : 3-Anhydro-4-*O*-methyl-	20	−25	−38	18·7	0·93

Demethylation of the syrup, R_G 0·90, gave a chromatographically pure syrup, R_G 0·72.

Application of the Dische test (Dische, *Mikrochem.*, 1930, **8**, 4; Deriaz, Stacey, Teece, and Wiggins, *J.*, 1949, 1222), as extended by Allerton, Overend, and Stacey (*J.*, 1952, 255) to 3-deoxyxylose and 2 : 3-dideoxyribose, to these dideoxy-derivatives gave values for the molecular extinction coefficients which were in reasonable agreement with those obtained by the last authors.

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GLUCOSAMINE 6-PHOSPHATE

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In 1951 Brown[1] reported the isolation of glucosamine 6-phosphate from the phosphorylation of glucosamine by A.T.P. in the presence of either brain or yeast hexokinase. The quantity isolated was small and attempts to isolate a crystalline hydrochloride were unsuccessful. Characterization was based on elementary analysis, reducing properties, acid stability, and the reaction with periodate. We have now prepared this substance by direct phosphorylation of glucosamine with metaphosphoric acid.[2] Dry glucosamine hydrochloride was stirred for several hours at 80° c. with metaphosphoric acid; acetonitrile was added to prevent excessive caramelization of the glucosamine. Removal of the acetronitrile and treatment of the chilled residue with *iso*propanol and anhydrous ether gave an insoluble material which was re-treated with metaphosphoric acid. The crude product was hydrolysed with N-hydrochloric acid at 100° to remove any phosphate groups on positions other than the 6-position of the sugar chain. Evaporation to dryness gave a dark-coloured residue (A) consisting of glucosamine and its 6-phosphate. Attempted separation on a column of anion exchange resin (Deacidite F) proved unsuccessful, condensation products being obtained. The residue (A) was washed with *iso*propanol to remove phosphoric and hydrochloric acids and, after solution of the residue in water, the last trace of phosphoric acid was precipitated as barium phosphate. The residual solution was concentrated and glucosamine-6-(barium phosphate) hydrochloride contaminated with glucosamine was precipitated by the addition of ethanol. A pure product was obtained only after repeated solution in water and precipitation with ethanol; it was a reducing, cream-coloured powder which was free from inorganic phosphate and had $[\alpha]_D^{18}$ + 24° (*c*, 0·7 in H_2O), $[\alpha]_D^{18}$ of the dipolar ion + 43° (*c*, 0·2 in H_2O, pH 2·50); *cf.* Brown[3] who records $[\alpha]_D^{24}$ of the dipolar ion + 48·5° (*c*, 0·51%, H_2O, pH 2·5). (Found: C, 17·2; H, 3·63; N, 2·9; P, 6·9; Ba 31·0. Calc. for $C_6H_{13}O_8NPClBa$: C, 16·7; H, 3·1; N, 3·2; P, 7·2; Ba, 31·8%). Chromatographic examination with *n*-propanol-conc. ammonia-water (6 : 3 : 1)[4] as eluant and 0·1% $FeCl_3 6H_2O$; 5% sulphosalicylic acid both in 80% ethanol[5] as spray revealed a single spot; aniline oxalate spray with glucose as control gave a single spot R_G 0·21 (glucose = 1·0); no evidence for the presence of glucosamine hydrochloride could be obtained. The uptake of sodium metaperiodate after 22 hours at room temperature in the dark (pH 4·5) was 2·8 mol. (70% of theory; 4 mol.), after 66 hours 3·7 mol. (92%) and after 100 hours 3·8 mol. (95%). Glucosamine hydrochloride under the same conditions consumed 4·7, 5·1 and 5·1 mol. respectively (theory 5 mol.)

Synthesis of this substance by an alternative route is also being carried out and full details of this and the above preparation will be published elsewhere.

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THE MERCAPTOLYSIS OF THE POLYSACCHARIDE FROM *CHONDRUS CRISPUS*

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Earlier work[1] on the polysaccharide from *Chondrus crispus* failed to identify a portion of the molecule which from its properties was thought to be an anhydro- or deoxy-sugar. Mercaptolysis experiments on the polysaccharide have now been carried out in order to verify this point. Araki and Hirase[2] isolated crystalline 3 : 6-anhydro-L-galactose diethylmercaptal from agar by this method, but, contrary to expectations, it was the D-form and not the L-form of this sugar which we obtained from the *Chondrus crispus* polysaccharide. It gave a positive Selivanoff test and had m.p. 110—111°, $[\alpha]_D^{18}$ — 12° (*c*, 2·0 in H_2O), + 21° (*c*, 2·3 in EtOH) (Araki and Hirase record m.p. 110—111°, $[\alpha]_D^{14}$ +14·09° in water and —21° in ethanol for 3 : 6-anhydro-L-galactose diethylmercaptal). (Found: C, 44·8; H, 7·3; S, 23·6. Calc. for $C_{10}H_{20}O_4S_2$: C, 44·75; H, 7·5; S, 23·9%). The corresponding 2 : 4 : 5-tri-*O*-*p*-nitrobenzoyl derivative has been prepared and has the correct constants.

The presence of L-galactose in this polysaccharide has also been confirmed by the isolation of DL-galactose diethylmercaptal, m.p. 127°, $[\alpha]_D^{20} \pm 0°$ (*c*, 0·64 in H_2O) (Found: C, 41·5; H, 7·5; S, 21·7. Calc. for $C_{10}H_{22}O_5S_2$: C, 41·9; H, 7·7; S, 22·3%).

Although the work on the detailed structure of the polysaccharide has not yet been completed it is considered desirable to publish these results in confirmation of the work of O'Neill[3] who has recently reported the isolation of the D-form of 3 : 6-anhydrogalactose diethylmercaptal from the mercaptolysis of the K-fraction of carrageenin.

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The Ammonolysis of Methyl 2 : 3-Anhydro-D-lyxofuranoside.

By J. M. ANDERSON and ELIZABETH PERCIVAL.

[Reprint Order No. 5857.]

BAKER, SCHAUB, JOSEPH, and WILLIAMS (*J. Amer. Chem. Soc.*, 1954, **76**, 4044) in the course of a total synthesis of puromycin reported the isolation of the α- and the β-form of methyl 3-acetamido-3-deoxy-D-arabofuranoside by ammonolysis, followed by *N*-acetylation, of the methyl 2 : 3-anhydro-D-lyxofuranosides. In investigations into the mode of fission of ethylene oxide rings we too carried out this series of reactions and isolated the two anomers. Baker *et al.* (*loc. cit.*) concluded that their product was the 3-amino-3-deoxy-D-arabinose derivative and not the other possible fission product, the 2-amino-2-deoxy-D-xylose derivative, inasmuch as the hydrochloride of their product had a different decomposition point and rotation from 2-amino-2-deoxy-D-xylose hydrochloride, prepared by Wolfrom and Anno (*J. Amer. Chem. Soc.*, 1953, **75**, 1038). We obtained proof that neither of the crystalline *N*-acetyl derivatives was a xylose derivative because all attempts to condense them with acetone were unsuccessful. The starting material was obtained in quantitative yield in every experiment, whereas 2-amino-2-deoxy-D-xylose would have given the 3 : 5-*iso*propylidene derivative. It is clear therefore that the main product from the fission of the oxide ring is an arabinose derivative.

Proof that no change to the pyranose form had occurred was obtained by the isolation from both anomers of the di-*O*-toluene-*p*-sulphonyl derivative followed by replacement of the primary toluene-*p*-sulphonyloxy-group by iodine. The action of sodium iodide in acetone was carried out under conditions specific for the replacement of a toluene-*p*-sulphonyloxy-group attached to a primary carbon atom (see Tipson, *Adv. Carbohydrate Chem.*, 1954, **8**, 192). Control experiments were carried out with methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside and toluene-*p*-sulphonamide. The former gave the same percentage yield of sodium toluene-*p*-sulphonate as did the above di-*O*-toluene-*p*-sulphonyl derivatives; the latter gave no sodium toluene-*p*-sulphonate, evidence that had the toluene-*p*-sulphonyl chloride condensed with the amino-group of the sugar it would not be removed under these conditions.

Experimental.—Solvents were removed under reduced pressure. Methanolic ammonia was prepared by saturating dry methanol with ammonia at 0°.

Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside. Methyl 2 : 3-anhydro-α-D-lyxofuranoside (*J.*, 1953, 564) (m. p. 80°; 0·90 g.), dissolved in dry methanolic ammonia (40 ml.), was heated in a sealed tube at 120° for 48 hr. After concentration of the cooled solution, distillation gave a viscous syrup (0·85 g.), b. p. 150—170°/0·01 mm. A solution of this in water (20 ml.) and methanol (2 ml.) was stirred at 5° with anion-exchange resin (Amberlite I.R. 400; bicarbonate form) (24 ml.) and acetic anhydride (0·6 ml.) for 90 min. (Roseman and Ludowieg, *J. Amer. Chem. Soc.*, 1954, **76**, 301). Filtration and evaporation gave a syrup (0·96 g.), which formed prisms (0·52 g.) from acetone, and on recrystallisation had m. p. 120—121°, $[\alpha]_D^{24}$ +124° (*c*, 0·7 in EtOH), +134° (*c*, 1·0 in H_2O) (Baker *et al.*, *loc. cit.*, record m. p. 115—116°, $[\alpha]_D$ +102° in H_2O) (Found : C, 47·2; H, 7·6; N, 6·5. Calc. for $C_8H_{17}O_5N$: C, 46·8; H, 7·4; N, 6·8%).

Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside. Syrupy methyl 2 : 3-anhydro-αβ-D-lyxoside (2·8 g.), in dry methanolic ammonia (75 ml.), was treated as described for the α-form. A portion (1·01 g.) of the derived syrup in dry methanol (20 ml.) was treated with acetic anhydride (2·5 ml.), and the mixture kept at 18° for 18 hr. This, after dilution with water (10 ml.), neutralisation with solid sodium hydrogen carbonate, filtration, and evaporation to dryness, gave a residue which was extracted with chloroform. Evaporation of the dried chloroform extract gave a syrup (0·76 g.) which, on solution in methanol–acetone (50%), addition of ether to incipient turbidity and recrystallisation, yielded needles (0·16 g.), m. p. 156°, $[\alpha]_D^{18}$ −165°

(*c*, 1·0 in EtOH), −120° (*c*, 1·2 in H_2O) (Baker *et al.*, record m. p. 155°, $[\alpha]_D$ −119° in H_2O) (Found: C, 46·2; H, 7·3; N, 6·9%).

Attempted isolation of an iso*propylidene derivative.* Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (50 mg.) was shaken with dry acetone (10 ml.), anhydrous copper sulphate (1 g.), and 2 drops of acetaldehyde for 14 days. This gave a syrup (45 mg.) which crystallised completely on trituration with acetone; the solid had m. p. and mixed m. p. with starting material 118—120°, $[\alpha]_D^{15}$ +124° (*c*, 0·7 in EtOH). Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside (50 mg.) was treated with dry acetone and anhydrous copper sulphate under the same conditions. The product (46 mg.) had m. p. and mixed m. p. with starting material 156°, $[\alpha]_D^{18}$ −160° (*c*, 0·5 in EtOH) (Found: C, 46·9; H, 7·35. Calc. for $C_8H_{17}O_5N$: C, 46·8; H, 7·4; for $C_{11}H_{19}O_5N$: C, 53·8; H, 7·8%).

*Methyl 3-acetamido-3-deoxy-2:5-di-O-toluene-*p*-sulphonyl-α-D-arabofuranoside.* Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (10 mg.) in dry pyridine (1 ml.) was treated with toluene-*p*-sulphonyl chloride (40 mg.) in the presence of "Drierite" for 60 hr. at room temperature. The toluene-*p*-sulphonyl derivative (11·7 μmoles) obtained after appropriate treatment, was dissolved in dry acetone and heated with sodium iodide in a sealed tube for 2 hr. at 100°. Characteristic plate-shaped crystals of sodium toluene-*p*-sulphonate (identified as the *S*-benzylthiouronium salt, m. p. 179—180°) were deposited in a yield (10·3 μmoles) comparable with that in a similar experiment carried out on methyl 2:3:4-tri-*O*-benzoyl-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside (9·3 μmoles from/11·4 μmoles). Toluene-*p*-sulphonamide failed to yield sodium toluene-*p*-sulphonate under similar treatment. Identical experiments were carried out with the β-anomer (10 mg.) and the derived 2:5-di-*O*-toluene-*p*-sulphonyl derivative (19·5 μmoles) on treatment with sodium iodide in acetone gave sodium toluene-*p*-sulphonate (16·5 μmoles). (Corrections for solubility of sodium toluene-*p*-sulphonate in acetone were made in all of these experiments.)

*3-Amino-3-deoxy-*D*-arabinose hydrochloride.* After methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (91 mg.) had been heated at 100° with hydrochloric acid (10 ml.; 3N) for 60 min. and the solution had been concentrated to dryness, a syrup was obtained which crystallised from methanol; the solid had m. p. 161° (decomp.), $[\alpha]_D^{18}$ −109° (*c*, 0·3 in H_2O) {Baker *et al.*, *loc. cit.*, record m. p. 159° (decomp.), $[\alpha]_D$ −112° in H_2O}.

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The Synthesis of Methyl Ethers of Mannuronic and Glucuronic Acid, and their Reaction with Periodate.

By R. A. Edington, E. L. Hirst, and Elizabeth E. Percival.

[Reprint Order No. 6119.]

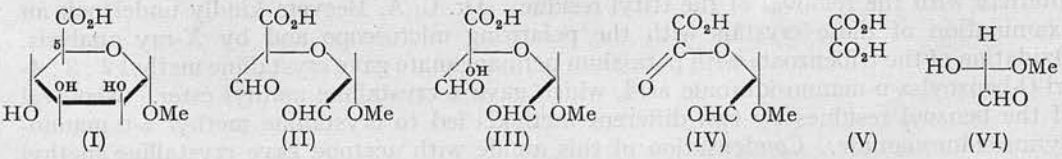
4-*O*-Methyl-D-mannuronic and 2 : 3-di-*O*-methyl-D-glucuronic acid have been synthesised for the first time and characterised by crystalline derivatives. 2 : 3 : 4-Tri-*O*-methyl-D-mannuronic acid has also been characterised as the crystalline methyl 2 : 3 : 4-tri-*O*-methyl-α-D-mannosiduronomethylamide. The action of periodate under varying conditions on these substances and on the two methyl ethers of D-galacturonic acid (Edington and Percival, *J.*, 1953, 2473) has been studied.

In continuation of our work on the synthesis of methyl ethers of D-galacturonic acid (Edington and Percival, *J.*, 1953, 2473) we have synthesised the 4-methyl ether of D-mannuronic acid and the 2 : 3-dimethyl ether of D-glucuronic acid. The first crystalline derivative of 2 : 3 : 4-tri-*O*-methyl-D-mannuronic acid has also been obtained. As the preparation of pure D-mannuronic acid derivatives from alginic acid proved exceedingly difficult it was considered preferable in the present work to prepare authentic crystalline derivatives of methyl α-D-mannoside and oxidise these to the uronic acid. Attempts to prepare crystalline methyl 2 : 3-*O*-*iso*propylidene-α-D-mannopyranoside (Ault, Haworth, and Hirst, *J.*, 1935, 517) and methyl 2 : 3-*O*-*iso*propylidene- and 2 : 3-*O*-benzylidene-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside were all unsuccessful. Accordingly crystalline methyl 2 : 3 : 4-*O*-tribenzoyl-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside (Haskins, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1946, **68**, 628) was utilised, and although the removal of the toluene-*p*-sulphonyl group by reduction (Kenner and Murray, *J.*, 1949, S178) could not be achieved this group was easily replaced by iodine; the latter was resistant to direct substitution by the hydroxyl group, but was easily replaced by nitrate. Reductive denitration then gave crystalline methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannoside. Parallel experiments on the successive treatment with triphenylmethyl chloride and benzoyl chloride of methyl α-D-mannopyranoside in pyridine gave methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannoside and this with hydrogen bromide in glacial acetic acid gave the same crystalline tribenzoate in high yield. As originally obtained or after recrystallisation from aqueous pyridine, methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannoside contained one molecule of firmly held pyridine of crystallisation and analogous compounds containing chloroform or acetone of crystallisation were obtained by recrystallisation from the corresponding solvent. The pyridine of crystallisation did not interfere with the removal of the trityl residue. Dr. C. A. Beevers kindly undertook an examination of these crystals with the polarising microscope and by *X*-ray analysis. Oxidation of the tribenzoate with potassium permanganate gave crystalline methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannosiduronic acid, which gave a crystalline methyl ester. Removal of the benzoyl residues by two different methods led to crystalline methyl α-D-mannopyranosiduronamide. Condensation of this amide with acetone gave crystalline methyl 2 : 3-*O*-*iso*propylidene-α-D-mannopyranosiduronamide and methylation with Purdie's reagents gave a crystalline product which was analysed as methyl 4-*O*-methyl-2 : 3-*O*-*iso*propylidene-α-D-mannosiduronomethylamide. The fact that the amide group had also been methylated was confirmed by conversion into the methyl ester and regeneration of the original substance by treatment with methylamine. This was further characterised by acid hydrolysis, followed by bromine oxidation and the isolation, as its crystalline diamide, of 4-*O*-methyl-D-mannaric acid (equivalent, by reason of the symmetry, to 3-*O*-methyl-D-mannaric acid).

Although 2 : 3 : 4-tri-*O*-methyl-D-mannuronic acid has been synthesised previously (Smith, Stacey, and Wilson, *J.*, 1944, 131; Ault, Haworth, and Hirst, *loc. cit.*) no crystalline derivative except the trimethylmannaramide was isolated. In the present work methylation with Purdie's reagents of methyl α-D-mannopyranosiduronamide gave crystalline methyl 2 : 3 : 4-tri-*O*-methyl-α-D-mannosiduronomethylamide. Again proof that the amide group had been methylated was obtained by hydrolysis and regeneration by treatment with methylamine. The regenerated material was further characterised, after hydrolysis and oxidation, as crystalline 2 : 3 : 4-tri-*O*-methyl-D-mannardiamide.

Although 2 : 3-di-*O*-methyl-D-glucuronic acid was isolated by Smith (*J.*, 1940, 1035) from methylated arabic acid no authentic synthesis of it has been reported. Methylation of methyl 4 : 6-*O*-benzylidene-α-D-glucoside (Freudenberg, Toepffer, and Andersen, *Ber.*, 1928, **61**, 1750) gave the crystalline 4 : 6-*O*-benzylidene-2 : 3-di-*O*-methyl derivative from which the benzylidene residue was removed by dilute acid at room temperature. Oxidation of the crystalline product with dilute aqueous alkaline permanganate, followed by esterification, led to syrupy methyl (methyl 2 : 3-di-*O*-methyl-α-D-glucopyranosid)uronate, which gave a crystalline *p*-nitrobenzoate identical (mixed m. p.) with a specimen prepared by Smith (*loc. cit.*) from the hydrolysate of methylated arabic acid. The crystalline phenylhydrazide of the 2 : 3-dimethyl ether was also prepared and had m. p. 195—197°. Smith (*loc. cit.*) records m. p. 225—227° for this derivative and we are grateful to him for a re-determination : his material had changed on storage to m. p. 207° (205° after resolidifying) and gave a mixed m. p. with our material of 196—200° (197° after resolidifying). The existence of two crystalline forms is not new in carbohydrate chemistry (cf. 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosamine, m. p. 144° and 126°; Smith *et al.*, *J. Amer. Chem. Soc.*, 1952, **74**, 1341). The dimethyl ether was further characterised by conversion into the known methyl 2 : 3 : 4-tri-*O*-methyl-α-D-glucosiduronamide and 2 : 3-di-*O*-methyl-D-glucaramide.

In spite of the fact that the original concept of specificity for the α-diol structure has been modified considerably, periodate remains a valuable tool. The presence of uronic acid residues in many polysaccharides makes it desirable, therefore, that precise information should be available concerning the reaction of these substances and their derivatives with periodate. Link and his co-workers (*J. Biol. Chem.*, 1945, **159**, 502) recorded that zinc bornyl glucosiduronate and methyl (methyl α-D-galactosid)uronate gave more than the theoretical amount of formic acid under the action of periodate. Halsall, Hirst, and Jones (*J.*, 1947, 1427) confirmed this and found that if they used potassium metaperiodate at pH 4 and kept the concentration of formic acid low the uronic acids underwent further oxidation. Link and his colleagues had explained the over-oxidation of the uronosides on the assumption that after the formation of the dialdehyde (II) the hydrogen situated on the original $C_{(5)}$ is activated by the adjacent carboxyl and aldehydic groups and is oxidised to a hydroxyl group (as in III). " This would result in the formation of a substance which in its hydrated form contains hydroxyl groups on adjacent carbon atoms and would undergo further oxidation with periodate with the formation of an ester of oxalic acid " (IV). At this stage the consumption of periodate is 4 mol. per mol. of uronic



acid. If the conditions are such that the ester (IV) is hydrolysed, the hemiacetal (VI) of glyoxal would be oxidised further and the total consumption of periodate would be 5 mol. Sprinson and Chargaff (*J. Biol. Chem.*, 1946, **164**, 435) investigated the conversion of the aldehyde (II) into the hydroxy-aldehyde (III) and proved that substances such as malonic acid which contain a hydrogen atom on a carbon atom situated between two carbonyl groups can be oxidised to the corresponding hydroxy-compound which then undergoes further oxidation.

In the present work methyl (methyl α-D-galactopyranosid)uronate was oxidised under different conditions of pH and temperature (see Table 1). Within 20 hours under all the conditions employed more than the 2 mol. of periodate required by the simple oxidation

TABLE 1. *Uptake of periodate (mol.) by methyl α-D-galactopyranosiduronic methyl ester.*

Temp. :	18°						0°		
Buffer :	None	A	B	C	D	E	None	B	E
pH :	—	2·0	4·5	5·3	5·3	7·0	—	4·5	7·0
Time (hr.)									
20	3·5	2·9	2·9	3·0	3·1	4·2	2·5	2·4	3·1
66	4·0	3·2	3·2	3·8	4·7	4·9	3·0	3·0	4·7
140	4·7	3·5	3·3	4·9	5·0	5·0	3·1	3·0	4·9
300	5·1	3·8	4·1	5·1	5·1	5·1	4·0	4·0	5·0

had been consumed. In 66 hours the consumption in all cases was 3 mol. or more and the reaction generally appeared to become slower after this point. In 300 hours reaction appeared to be complete at a consumption of 5·0—5·1 mol. except in four cases, notably at pH 4·5 at 0° and 18°, in which the consumption was still only 4·0—4·1 mol. Meyer and Rathgeb (*Helv. Chim. Acta*, 1949, **32**, 1102) state that, if the oxidation is carried out at 0° and the pH kept between 5·7 and 4·2, then the hydrolysis of formyl esters is negligible. It appears probable therefore that the oxidations carried out at pH 4·5 are arrested at the ester (IV). From the results it appears that some overoxidation occurs under all the conditions studied and that, apart from the figure of 5 mol. representing complete overoxidation, the only significant arrest in the progress of oxidation occurs at the α-hydroxy-aldehyde (III) after the consumptiom of 3·0 mol. of oxidant. According to Halsall, Hirst, and Jones (*loc. cit.*) this is to be expected as the rate of oxidation of the α-hydroxy-aldehyde is comparatively slow.

Of the four glycosiduronic acid derivatives studied (see Table 2) in which overoxidation

TABLE 2. *Uptake of periodate (mol.) by uronic acid derivatives.*

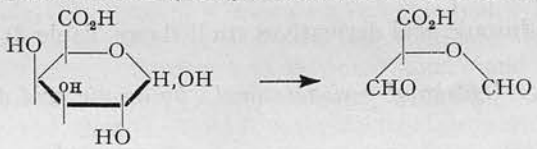
	Mol. expected			Buffer B pH 4·5, 0°		Buffer E pH 7·0, 18°	
Substance	(*a*)	(*b*)		90 hr.	300 hr.	90 hr.	300 hr.
Me (Me α-D-galactopyranosid)uronate	2	5		3·0	4·0	5·0	5·1
Me α-D-galactopyranosiduronamide	2	5		2·3	2·5	2·8	4·7
Me α-D-mannopyranosiduronamide	2	5		2·3	2·7	2·8	4·7
Me (Me 2-*O*-methyl-α-D-galactosid)uronate	1	3		1·4	2·0	2·0	3·0
Me (Me 4-*O*-methyl-α-D-mannosid)uronate	1	1		0·9	1·0	1·0	1·1
Me (Me 3 : 4-di-*O*-methyl-α-D-galactosid)uronate	0	0		0·0	0·0	0·0	0·0
Me (Me 2 : 3-di-*O*-methyl-α-D-glucosid)uronate	0	0		0·0	0·0	0·0	0·0
Me (Me 2 : 3 : 4-tri-*O*-methyl-α-D-glucosid)uronate	0	0		0·0	0·0	0·0	0·0
Me 2 : 3 : 6-tri-*O*-methyl-α-D-mannosiduronomethylamide	0	0		0·0	0·0	0·0	0·0
	(*c*)	(*d*)	(*e*)				
D-Galacturonic acid	5	3	5	3·3	3·6	—	—
D-Mannuronic acid	5	3	5	3·9	4·2	—	—
4-*O*-Methyl-D-mannuronic acid	3	2	2	2·0	2·1	—	—
2-*O*-Methyl-D-galacturonic acid	3	1	3	1·5	1·9	—	—
3 : 4-Di-*O*-methyl-D-galacturonic acid	2	1	1	1·1	1·2	—	—
2 : 3-Di-*O*-methyl-D-glucuronic acid	2	0	0	0·9	1·2	—	—
2 : 3 : 4-Tri-*O*-methyl-D-glucuronic acid	1	0	0	0·0	0·0	—	—

(*a*) Without overoxidation. (*b*) With overoxidation. (*c*) Open-chain configuration. (*d*) Pyranose, without overoxidation. (*e*) Pyranose, with overoxidation.

was expected it did occur, but the numerical results are difficult to reconcile with theory. At 0° and pH 4·5 the overoxidation was small even after 300 hours, methyl α-D-galacto-siduronamide and mannosiduronamide consuming respectively 2·5 and 2·7 mol. of periodate instead of the postulated 5 mol. Methyl (methyl 2-*O*-methyl-α-D-galactosid)uronate consumed 2 mol., mid-way between normal oxidation and complete overoxidation. No over-oxidation occurred with methyl (methyl 4-*O*-methyl-α-D-mannosid)uronate; on the current

theories this is to be expected as the 4-methyl group would prevent oxidation between positions 3 and 4 and consequently the formation of an active hydrogen atom. This is also in agreement with the results of Smith (*J.*, 1951, 2646) who found that methyl (methyl 4-*O*-methyl-α-D-glucosid)uronate consumed only 1 mol. of periodate. With two dimethyl- and two trimethyl-uronosidic methyl esters where no oxidation of any kind was expected the results fitted expectations.

A number of free uronic acids were oxidised at 0° and pH 4·5 (see Table 2); under these conditions, if a formic ester is produced, it is unlikely to be hydrolysed (Meyer and Rathgeb, *loc. cit.*). The periodate consumption after 300 hours was in every case about 1 mol. less than would be expected if the uronic acid reacted in the straight-chain (aldehydo-)form. In every experiment the glycosiduronic ester was hydrolysed with N-sulphuric acid at 100° for 24 hours and no attempt was made to isolate the product before oxidation with periodate and it might be argued that low consumption of periodate was due to loss of sugar during hydrolysis. However, hydrolysis of methyl (methyl 3 : 4-di-*O*-methyl-α-D-galactosid)-uronic under the same conditions gave a 97% yield of the free acid. White (*J. Amer. Chem. Soc.*, 1953, **75**, 4692) found that 3 : 4-di-*O*-methyl-D-glucuronic acid consumed only 1 mol. of periodate which is in agreement with the present results for 3 : 4-di-*O*-methyl-D-galacturonic acid. Greville and Northcote (*J.*, 1952, 1945) found that 3 : 4-di-*O*-methyl-D-glucose consumed only 1 instead of 2 mol. of periodate which could be explained by assuming that the sugar reacts in the pyranose configuration, although these authors consider that the immunity of $C_{(5)}$ and $C_{(6)}$ to oxidation may only partly be due to the impossibility of the sugar's assuming the furanose configuration. Application of this concept to the uronic acids oxidised in the present work gives figures which fit experimental results in the case of 4-*O*-methyl-D-mannuronic acid, 3 : 4-di-*O*-methyl-D-galacturonic acid, and 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. For the remaining uronic acids it must be borne in mind that if a uronic acid having 3- and 4-hydroxyl groups reacts in the pyranose form one of the intermediates contains activated hydrogen and overoxidation is therefore to be expected.



This overoxidation would be expected with D-galacturonic, D-mannuronic, and 2-*O*-methyl-D-galacturonic acid, in all of which the actual periodate consumption exceeds the figure predicted on the basis of pyranose configuration without overoxidation. 2 : 3-Di-*O*-methyl-D-glucuronic acid also consumes more periodate than would be expected from the pyranose form although in this case overoxidation due to activated hydrogen is hardly to be expected.

Experimental

All solvents were removed under reduced pressure and below 50°. M. p.s were determined on the Kofler hot-stage microscope. Silver and barium salts were removed by filtration through layers of charcoal and Celite and the residue on the filter washed at least thrice with a suitable warm solvent. Optical rotations were determined at 18° ± 2° in $CHCl_3$ unless otherwise stated. Light petroleum was the fraction of b. p. 60—80°.

Methyl 2 : 3 : 4-*Tri*-O-*benzoyl*-6-O-*triphenylmethyl*-α-D-*mannoside*.—Methyl α-D-mannopyranoside, m. p. 194—195° (105·5 g.), isolated from carob gum (supplied by Messrs. Ellis Jones, Stockport) (Smith, *J.*, 1948, 1989) was heated with triphenylmethyl chloride (175 g.) and dry pyridine (1050 ml.) at 50° with occasional shaking, until all the solid had dissolved (6 hr.), then kept at room temperature for 18 hr., after which benzoyl chloride (230 ml.) was added rapidly without cooling. The mixture was set aside at room temperature for 24 hr. and the crystalline solid filtered off, and washed with pyridine (100 ml.), with ethanol (2 × 100 ml.), with water (3 l.), and again with ethanol (2 × 100 ml.). After drying, the *product* was a colourless crystalline solid (368 g., 82%), m. p. 100—120°, $[\alpha]_D$ −110° (Found : C, 75·1; H, 5·3; N, 1·9. $C_{47}H_{40}O_9,C_5H_5N$ requires C, 75·4; H, 5·5; N, 1·7%). Recrystallisation from aqueous or ethanolic pyridine gave an unchanged product. Recrystallisation from acetone gave stout prisms, m. p. 100—115°, $[\alpha]_D$ −114° (Found : C, 74·2; H, 6·1. $C_{47}H_{40}O_9,C_3H_6O$ requires C, 74·3; H, 5·7%). Recrystallisation from ethanol–chloroform gave plates, m. p. 100—115°, $[\alpha]_D$ −107° (Found : C, 67·9;

H, 5·0; Cl, 9·3; loss of wt. at 100°/15 mm. in 15 hr., 10·4. $4C_{47}H_{40}O_9,3CHCl_3$ requires C, 68·4; H, 4·9; Cl, 9·5; $CHCl_3$, 10·7%). The crystals from each of these solvents, after melting, recrystallised between 115—125° and had a final m. p. 188—189°. After removal of the solvent *in vacuo* the *product* was a fine white powder, m. p. 189—191°, $[\alpha]_D$ −121° (Found: C, 75·4; H, 5·3. $C_{47}H_{40}O_9$ requires C, 75·4; H, 5·4%).

Examination with the polarising microscope [Dr. BEEVERS]. Crystals from $CHCl_3$: flat prisms showing an extinction at 45° to their length. Crystals from acetone: prisms, some with parallel and some with a 45° extinction. Crystals from aqueous pyridine: small prisms, a woolly mass, 22° extinction. Crystals from $CHCl_3$–EtOH: opaque mass, no single crystals visible. Conclusion: These crystals are of low symmetry, and perhaps show more than one crystal form.

X-*Ray Examination* [Dr. BEEVERS]. Oscillation and Weissenberg photographs (zero and first layer-line) were taken of two crystals from chloroform. Both specimens showed a similar phenomenon, a doubling of the spots, more pronounced in some directions than in others, indicating a twinning of two slightly different lattices very similar to one another. A detailed analysis of the two lattices has not been made, although it is thought that both are either monoclinic or triclinic. If the doubled spots are treated as one, an "average" lattice can be deduced, and has the following properties: monoclinic, $a = 9{\cdot}07$, $b = 24{\cdot}3$, $c = 10{\cdot}4$ Å, $\beta = 105°\ 27'$. This cell is body-centred (though of course it can be transformed into an A or a C face-centred lattice). The volume of the cell is 2209 Å^3, and this value gives 2·05 of (molecule + $\frac{3}{4}CHCl_3$) per cell. Since this refers to an "average" cell it is quite possible that there are 2 molecules of chloroform in one kind of cell and 1 in the other, although it might also be that there are 1·5 molecules in both kinds of cell. The two cells appear to be equally numerous, *i.e.*, the average intensity of the two sets of spots is about the same.

It is hoped that an opportunity may arise for the lengthy detailed study of these twins, especially as a large single crystal of the product from acetone is available.

Methyl 2 : 3 : 4-*Tri*-O-*benzoyl*-6-O-*toluene*-p-*sulphonyl*-α-D-*mannoside*.—Methyl α-D-mannoside (107 g.) was stirred with dry pyridine (1000 ml.) for 30 min. at room temperature, then cooled to 0°, and a solution of toluene-*p*-sulphonyl chloride (112 g., 1·05 equiv.) in pyridine (250 ml.) was added with stirring and cooling to 0° during 9 hr. After a further hour's stirring at room temperature the mixture was cooled to 0° and water (1000 ml.) added with vigorous stirring during 20 min. More water (1500 ml.) was added and the solution extracted with chloroform (4 × 600 ml.). The extracts were washed with water and dried. Removal of the solvent gave a yellow syrup (146 g., 76%) (Found: S, 9·2; $C_7H_7O_3SNa$, 54·3. $C_{14}H_{21}O_8S$ requires S, 9·2; $C_7H_7O_3SNa$, 55·8%). The syrup (146 g.) in pyridine (400 ml.) was treated with benzoyl chloride (258 ml., 4 equiv.) and set aside at room temperature for 24 hr. After treatment with water (20 ml.) the mixture was poured into saturated aqueous sodium hydrogen carbonate, a red gum separating. Repeated extraction under reflux with ethanol left a white residue of tribenzoate which recrystallised from chloroform–ethanol as colourless plates (157 g., 50% from methyl α-D-mannoside), m. p. 198°, $[\alpha]_D$ −104° (Haskins, Hann, and Hudson, *loc. cit.*, record 38% yield and m. p. 197—199°, $[\alpha]_D$ −102°) (Found: C, 63·5; H, 4·9; S, 5·1. Calc. for $C_{35}H_{32}O_{11}S$: C, 63·6; H, 4·9; S, 4·9%).

Methyl 2 : 3 : 4-*Tri*-O-*benzoyl*-α-D-*mannoside*.—(*a*) Methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannoside (20 g.) was shaken vigorously for 90 sec. with hydrogen bromide (10%; w/v) in glacial acetic acid (33 ml.) and filtered immediately into a mixture of water (1500 ml.) and chloroform (1000 ml.). Fourteen further portions (each 20 g.) were treated in the same way and all were filtered into the same chloroform–water mixture. The aqueous layer was extracted with chloroform (2 × 400 ml.) and the combined chloroform extracts, after being washed and dried, gave a crystalline solid on evaporation. When an ethanol solution of this solid was kept overnight at room temperature crystalline starting material (5 g.; m. p. 95—120°, 187—189°) was deposited. Evaporation of the mother-liquors gave a crystalline *tribenzoylmannoside* (172 g., 95%), which after recrystallisation from aqueous alcohol had m. p. 143—145°, $[\alpha]_D$ −160° (Found: C, 66·6; H, 5·3. $C_{28}H_{26}O_9$ requires C, 66·4; H, 5·2%).

(*b*) The foregoing toluene-*p*-sulphonate was recovered unchanged and in good yield after hydrogenation with Raney nickel in ethyl acetate at 1 and 3 atm. during 72 hr. with shaking at 20°, at 100 atm. during 12 hr. with stirring, and under the conditions recorded by Mozingo *et al.* (*J. Amer. Chem. Soc.*, 1945, **67**, 2092) for 6 hr. at 77° with or without a stream of hydrogen. In a final experiment at 100°/100 atm. for 12 hr. a non-reducing syrup, $[\alpha]_D$ ±0°, was isolated (Found: C, 63·0; H, 7·4; S, 2·4; OMe, 6·7%; equiv., 184). It gave a negative test for primary toluene-*p*-sulphonyl ester (Tipson, Clapp, and Cretcher, *J. Org. Chem.*, 1947, **12**, 133).

(*c*) The toluene-*p*-sulphonate (6 g.) was converted into the 6-deoxy-6-iodo-derivative according to Oldham and Rutherford's method (*J. Amer. Chem. Soc.*, 1932, **54**, 366). Recrystallisation from acetone gave large prisms of the 6-iodide (5·05 g., 90%), m. p. 199—201°, $[\alpha]_D$ −106° (Found: C, 55·1; H, 4·1; I, 20·6. Calc. for $C_{28}H_{25}O_8I$: C, 54·5; H, 4·1; I, 20·6%). Treatment of this in dry benzene with dry or with moist silver oxide with vigorous shaking for 7 days, followed by filtration and evaporation, gave the starting material in quantitative yield. Heating with moist silver oxide in benzene in a sealed tube at 100° for 2 hr. gave, after filtration and evaporation, a dark, reducing syrup.

The iodide (13 g.), in acetonitrile (200 ml.), was heated under reflux for 4 hr. with powdered silver nitrate (4 g.). Excess of silver nitrate was removed by treating the cooled solution with sodium iodide (1·0 g.) in acetone. After removal of the precipitated silver iodide the solution was diluted with chloroform (300 ml.) and extracted with water (3 × 300 ml.). Evaporation of the dried chloroform layer gave crystals from which unchanged starting material (6·2 g.; m. p. 193°) was removed by fractional crystallisation from ethanol. The more soluble *nitrate* was obtained from 90% ethanol as large prisms (5·5 g., 91%), m. p. and mixed m. p. with a specimen provided by Dr. G. O. Aspinall (prepared by direct nitration of methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannoside, unpublished work) 103—104°, $[\alpha]_D$ −116° (Found: C, 61·1; H, 4·5; N, 2·5. $C_{28}H_{25}O_{11}N$ requires C, 61·0; H, 4·6; N, 2·5%). A solution of this nitrate (2·0 g.) in glacial acetic acid (10 ml.) and benzene (20 ml.) was treated at room temperature with equal quantities of zinc and iron powders until the solution no longer gave a pink colour with diphenylbenzidine in concentrated sulphuric acid. Filtration and extraction with benzene, followed by aqueous washing of the benzene extracts and evaporation, gave crystals (1·3 g., 71%), m. p. 143° alone or mixed with the previous end-product (after recrystallisation from ethanol).

Methyl α-D-*Mannopyranosiduronamide.*—A solution of methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannoside (150 g.) in acetone (1500 ml.), glacial acetic acid (1500 ml.), and water (150 ml.) was stirred at room temperature, and potassium permanganate (160 g.; "AnalaR") was added in small portions during 30 hr. (Stacey, *J.*, 1939, 1529). After a further 18 hr. the solution was decolorised by aqueous potassium metabisulphite. 4N-Sulphuric acid (150 ml.) was added, and chloroform extraction (3 × 1000 ml.) followed by evaporation gave *methyl* 2 : 3 : 4-*tri*-*O*-*benzoyl*-α-D-*mannosiduronic acid* (131 g., 85%) which crystallised on storage and after recrystallisation from chloroform–light petroleum had m. p. 180—181·5°, $[\alpha]_D$ −140° (Found: C, 64·5; H, 4·7. $C_{28}H_{24}O_{10}$ requires C, 64·6; H, 4·6%). Treatment with methanolic hydrogen chloride (0·5%) at 20° for 72 hr. led to crystalline methyl (methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannosid)-uronate (123 g., 92%) which after recrystallisation from methanol had m. p. 143—144°, $[\alpha]_D$ −127° (Found: C, 65·2; H, 4·9. $C_{29}H_{26}O_{10}$ requires C, 65·1; H, 4·9%). This ester (100 g.) was dissolved in saturated methanolic ammonia (1100 ml.) and set aside at room temperature for 24 hr. Evaporation gave a syrup from which benzamide was removed by repeated extraction with ether (250-ml. portions). The crystalline residue of *methyl* α-D-*mannopyranosiduronamide* was washed once with acetone and recrystallised from aqueous acetone as colourless prisms (20·1 g., 52%), m. p. 182—183°, $[\alpha]_D^{18}$ +66° (*c*, 1·1 in H_2O) (Found: C, 41·0; H, 6·3; N, 6·6. $C_7H_{13}O_6N$ requires C, 40·6; H, 6·3; N, 6·7%). Removal of the benzoyl groups from the previous tribenzoate with sodium methoxide (Zemplén and Pacsu, *Ber.*, 1929, **62**, 1613), followed by esterification with methanolic hydrogen chloride (2%), gave syrupy methyl (methyl α-D-mannopyranosid)uronate, n_D^{19} 1·4842, $[\alpha]_D^{22}$ +80° (*c*, 0·5 in H_2O); the derived amide (45% yield from the tribenzoate ester) had m. p. and mixed m. p. 182—183°, $[\alpha]_D^{15}$ +63° (*c*, 0·9 in H_2O).

Methyl (*Methyl* 4-O-*Methyl*-α-D-*mannosid*)*uronate.*—The foregoing amide (20 g.) was condensed with acetone (750 ml.) (Percival and Percival, *J.*, 1950, 690). After 4 days the supernatant liquid was decanted off and neutralised with barium carbonate, and the residual solids were shaken for a further 2 days with acetone (500 ml.) containing sulphuric acid (0·03% v/v). Condensation of the residual solids with acetone was repeated a further 6 times after which the barium salts were removed from the combined neutral extracts. Evaporation of the acetone gave crystalline *methyl* 2 : 3-O-iso*propylidene*-α-D-*mannosiduronamide*, which recrystallised from acetone–light petroleum as large colourless prisms (15·0 g., 63%), m. p. 177·5—179°, $[\alpha]_D$ +14° (*c*, 1·0 in H_2O) (Found: C, 49·0; H, 6·8; N, 5·1. $C_{10}H_{17}O_6N$ requires C, 48·5; H, 6·9; N, 5·7%). Threefold methylation with methyl iodide and silver oxide gave *methyl* 4-O-*methyl*-2 : 3-O-iso*propylidene*-α-D-*mannosiduronomethylamide* (1·09 g. from 1 g.; 93%) which crystallised and after recrystallisation from light petroleum had m. p. 151—153°, $[\alpha]_D^{18}$ +23° (*c*, 0·6 in H_2O) (Found: C, 52·4; H, 7·7; N, 5·3. $C_{12}H_{21}O_6N$ requires C, 52·4; H, 7·6; N, 5·1%). Hydrolysis with 2N-sodium hydroxide at 100° for 3 hr., followed by esterification of the resulting

syrup with ethereal diazomethane and then treatment with ethanolic methylamine, regenerated the crystalline methylamide. This methylamide (2·0 g.) was heated at 100° for 30 min. with N-sodium hydroxide (20 ml.), then cooled to −5° and acidified with 4N-sulphuric acid (7 ml.); the solution was extracted with chloroform (4 × 50 ml.); after being washed with water the chloroform extracts were evaporated to a syrup which was dissolved in 1% methanolic hydrogen chloride and kept at room temperature for 48 hr. Appropriate treatment gave *methyl* (*methyl* 4-O-*methyl*-α-D-*mannosid*)*uronate* as a syrup (1·5 g.), n_D^{17} 1·4727, $[\alpha]_D^{19}$ +84° (*c*, 1·0 in H_2O) (Found: C, 45·3; H, 7·3. $C_9H_{16}O_7$ requires C, 45·8; H, 6·8%). Methylation twice with methyl iodide and silver oxide, followed by treatment with ethanolic methylamine, gave methyl 2 : 3 : 4-tri-*O*-methyl-α-D-mannosiduronomethylamide, m. p. 103—105° alone or mixed with a specimen prepared by direct methylation of methyl α-D-mannopyranosiduronamide. After hydrolysis of the preceding ester (0·097 g.) with N-hydrochloric acid at 100° for 24 hr. in a sealed tube, oxidation with bromine water (7 days at 40°), esterification, distillation (b. p. 160—170°/0·1 mm.) and treatment with methanolic ammonia gave 2 : 3 : 4-*tri*-O-*methyl*-D-*mannardiamide* (0·030 g.) m. p. 192—193°, $[\alpha]_D^{15}$ −16° (*c*, 0·6 in H_2O) (Found: C, 38·0; H, 6·2; N, 12·5. $C_7H_{14}O_6N_2$ requires C, 37·8; H, 6·4; N, 12·6%).

Methyl 2 : 3 : 4-*Tri*-O-*methyl*-α-D-*mannosiduronomethylamide*.—Methyl α-D-mannopyranosiduronamide (1·99 g.) was methylated thrice with methyl iodide (23 ml.) and silver oxide (12 g.), dioxan being added as a solvent in the first operation. A colourless syrup (2·618 g., 103%), n_D^{15} 1·4661, which crystallised on trituration with light petroleum was obtained. After recrystallisation from light petroleum the *methylamide* had m. p. 103—105°, $[\alpha]_D^{19}$ +42° (*c*, 1·4 in H_2O) (Found: C, 50·4; H, 8·0; N, 4·8. $C_{11}H_{21}O_6N$ requires C, 50·2; H, 8·1; N, 5·3%). Hydrolysis with sodium hydroxide, esterification, and treatment with ethanolic methylamine regenerated it in good yield. Treatment of the methylamide (0·10 g.) with 2N-sodium hydroxide, then with N-hydrochloric acid, oxidation with bromine water, esterification, distillation of the derived ester (b. p. 130—140°/0·1 mm.), and treatment with methanolic ammonia gave the trimethylmannardiamide (0·030 g.), m. p. 211° (decomp.) unchanged by repeated recrystallisation from methanol or on admixture with a synthetic specimen prepared by Haworth, Hirst, Isherwood, and Jones (*J.*, 1939, 1878) which was now found to have m. p. 211° on the Kofler apparatus and in a capillary tube.

Methyl 2 : 3-*Di*-O-*methyl*-α-D-*glucopyranoside*.—Methyl α-D-glucopyranoside, m. p. 163—165° $[\alpha]_D^{17}$ +158° (*c*, 1·8 in H_2O) (100 g.), was converted into the 4 : 6-*O*-benzylidene derivative according to the method of Freudenberg *et al.* (*loc. cit.*). The crude product was extracted with boiling water (1500 ml.), and the aqueous extract filtered whilst hot. On cooling, the product was obtained as long needles (102 g., 70%), m. p. 164—165°, $[\alpha]_D$ +108° (Found: C, 59·0; H, 6·6. Calc. for $C_{14}H_{18}O_6$: C, 59·6; H, 6·4%). A solution of it (50 g.) in acetone (300 ml.) was methylated twice with aqueous 30% sodium hydroxide (320 ml.) and methyl sulphate (130 ml.) (Bell, *J.*, 1936, 859). Methyl 4 : 6-*O*-benzylidene-2 : 3-di-*O*-methyl-α-D-glucoside (51·5 g., 94%) had, after recrystallisation from ethanol, m. p. 121—122°, $[\alpha]_D$ +95° (Found: C, 61·8; H, 7·0. Calc. for $C_{16}H_{22}O_6$: C, 61·9; H, 7·2%). A solution of this (38·4 g.) in acetone (800 ml.) and 4N-sulphuric acid (200 ml.) was kept at room temperature until the rotation of the solution became constant, $[\alpha]_D$ +110° (72 hr.). After neutralisation (barium carbonate) the combined filtrate and washings were concentrated to a syrup from which benzaldehyde was removed by repeated distillation with water. The residue of methyl 2 : 3-di-*O*-methyl-α-D-glucopyranoside (23·5 g., 86%), recrystallised from carbon tetrachloride, had m. p. 81—82°, $[\alpha]_D^{18}$ +153° (*c*, 1·0 in H_2O) (Found: 48·9; H, 8·0. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·1%).

Methyl (*Methyl* 2 : 3-*Di*-O-*methyl*-α-D-*glucopyranosid*)*uronate*.—The last mentioned glucoside (27·3 g.) dissolved in aqueous potassium hydroxide (14 g. in 2 l.) was stirred at room temperature and potassium permanganate (37 g.) added in small portions during 8 hr.; stirring was continued for a further 16 hr. Excess of permanganate was decomposed by aqueous potassium metabisulphite, the mixture filtered, and the residue washed with hot water (4 × 150 ml.). The combined filtrate and washings were neutralised with solid carbon dioxide and evaporated to dryness. The resultant white solid was extracted with ether (2 × 300 ml.) and then with boiling ethanol (3 × 300 ml.), and the ethanolic extracts were passed through a column (17 × 700 mm.) of Amberlite resin IR-120. The column was washed with water (200 ml.) and evaporation of the combined eluate and washings gave a syrup (20·5 g.) which was converted into the ester by treatment with 1% methanolic hydrogen chloride (500 ml.) at room temperature for 48 hr. Distillation of the product gave methyl (methyl 2 : 3-di-*O*-methyl-α-D-glucopyranosid)uronate as a syrup (20·9 g., 68%), b. p. 130—140°/0·5 mm., n_D^{18} 1·4441, $[\alpha]_D^{18}$ +111° (*c*, 1·1 in H_2O). The derived 4-*O*-*p*-nitrobenzoyl derivative (Smith, *J.*, 1940, 1035) (3·5 g. from

2·2 g.) had m. p. 156—158° alone or mixed m. p. with a specimen kindly supplied by Professor F. Smith, $[\alpha]_D$ +69° (Found: C, 51·4; H, 5·4; N, 4·1. $C_{17}H_{21}O_{10}N$ requires C, 51·1; H, 5·3; N, 3·5%). The derived uronophenylhydrazide, isolated as a crystalline residue (1·85 g.) after the ester (2·0 g.) had been heated with freshly distilled phenylhydrazine (0·78 ml.) in carbon dioxide in a sealed tube at 110° for 18 hr. and extracted with ether (3 × 20 ml.) under reflux, had m. p. 195—197° (from benzene), $[\alpha]_D$ +85°. Smith (*loc. cit.*) records m. p. 225—227°. The mixed m. p., kindly determined by Professor F. Smith with his specimen, was 196—200°, 197° (after resolidification) (Found: C, 55·2; H, 6·8; N, 9·0. Calc. for $C_{15}H_{22}O_6N_2$: C, 55·2; H, 6·8; N, 8·6%). Hydrolysis of the methyl uronate with N-hydrochloric acid at 100° for 24 hr., followed by oxidation with bromine water, esterification, and distillation, gave syrupy 2 : 3-di-*O*-methyl-D-glucaro-l→4-lactone 6-methyl ester, b. p. 120—130°/0·1 mm., which crystallised after distillation and on recrystallisation from benzene had m. p. 99—100°, $[\alpha]_D^{18}$ +17° (*c*, 1·2 in H_2O). The derived amide had m. p. and mixed m. p. 154—155°. Two Purdie methylations of the methyl uronate (70 mg.), distillation, amide formation, and recrystallisation from benzene, gave 2 : 3 : 4-tri-*O*-methyl-α-D-glucosiduronamide, m. p. and mixed m. p. 180°, $[\alpha]_D^{15}$ +147° (*c*, 0·7 in H_2O).

Oxidation by Periodate.—Buffer solutions were prepared according to Vogel ("Quantitative Inorganic Analysis," Longmans Green and Co., London, 1939, p. 808), except that sodium salts were used in all experiments. The following buffer solutions were used (total molarity ~ 0·1M): A (pH 2·0), toluene-*p*-sulphonic acid–sodium toluene-*p*-sulphonate. B (pH 4·5), acetic acid–sodium acetate. C (pH 5·3), acetic acid–sodium acetate. D (pH 5·3), disodium hydrogen phosphate–sodium dihydrogen phosphate. E (pH 7·0), sodium dihydrogen phosphate–sodium hydroxide.

A typical oxidation is described in detail. The weighed substance (10—50 mg.; sufficient to give a back-titration difference of 10—20 ml.) was dissolved in about 40 ml. of the buffer solution at the correct temperature. 0·097M-Sodium metaperiodate (5 ml.) was added, the volume made up to 50 ml. with buffer solution, and the whole set aside in the dark at the appropriate temperature. At suitable intervals, portions of 10 ml. were saturated with sodium hydrogen carbonate, and 10 ml. of 0·1N-sodium arsenite and 1 g. of potassium iodide were added. This mixture was then set aside for 15 min. and finally titrated quickly with 0·1N-iodine (starch) until addition of 1 drop of iodine gave a blue colour which persisted for 5 sec. with shaking. A control experiment was conducted similarly. The detailed results for methyl α-D-galactopyranosiduronic methyl ester are given in Table 1 and the summarised results for all the derivatives oxidised are given in Table 2.

The authors are grateful to Dr. C. A. Beevers for examining the crystals of methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannoside with the polarising microscope and by *X*-rays, and to Mr. H. Peglar for the preparation of some of the methyl α-D-mannoside from carob gum. They thank the University of Edinburgh for the award of a Vans Dunlop Scholarship, the Department of Scientific and Industrial Research for the award of a maintenance grant to one of them (R. A. E.), and the Distillers Company Limited for a grant.

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WATER SOLUBLE POLYSACCHARIDE OF *Cladophora rupestris*

By I. S. Fisher and E. E. Percival (Scotland)

Read by E. E. Percival

Cladophora rupestris, a green seaweed with a high cellulose and protein content, was reported by H. Kylin[1] in 1944 to give a slimy water-soluble material which contained galactose and which was an ethereal sulphate of calcium and magnesium. As no other work has been published on the water-soluble polysaccharide of this weed it appeared highly desirable to investigate it further.

We are indebted to the Scottish Institute of Seaweed Research for the collection of the weed in November, 1953, from the East Coast of Scotland near Dunbar, between high and low tide. The weed was first freed from extraneous matter by washing with cold running water, and, after removal of some of the colouring matter by several treatments with cold alcohol, the polysaccharide was extracted with boiling water or with dilute hydrochloric acid (pH 3–4) at 70° C. Concentration of the extracts, under reduced pressure at 45° C, gave a viscous solution from which the polysaccharide was isolated, both by freeze-drying and by precipitation in alcohol, in an overall yield from the dried weed of 11·4%. This material had a nitrogen content of 3·0% which represents a protein content of 19·5%, and numerous attempts were made to purify it[2–11]. The most satisfactory and economical method was partial precipitation of the protein with 4% trichloroacetic acid, followed by fractional precipitation of the polysaccharide with alcohol. After removal of impure fractions with 50 and 70% alcohol, a material was precipitated with 90% alcohol, in approximately 5% yield by weight from the dried weed with $N=1{\cdot}2\%$. All subsequent work was carried out on this material, hereinafter called polysaccharide A. It was an off-white powder, $[a]_D^{20}+69°$, it was non-reducing and contained no ketose, amino sugar or uronic acid residue. It had OMe=0; $CH_3CO=0$, Ash, 13·7%, ash (as SO_4'') 16·7%, which had the composition SO_4 62·0%, Ca^{++} 22·1,

Fe^{+++} 4·4, Na^{+} 3·7, K^{+} 0·4, and an insoluble residue ($As_2O_3 + SiO_2$) 6·1%. While cations such as Ca^{++} could be precipitated, no SO_4'' could be detected in solutions of the polysaccharide until after hydrolysis. Furthermore the sulphate content of polysaccharide A was 19·6%, and that calculated from the ash 9·9%, a ratio of 2 : 1 which is characteristic of polysaccharide ethereal sulphates where:

$$2RO \,.\, SO_2 \,.\, ONa \xrightarrow{\text{heat}} Na_2SO_4 + SO_3 + \text{products of combustion}$$
(R=sugar residue)

and this polysaccharide is therefore a sulphuric ester similar to the polygalactan sulphuric ester of *Chondrus crispus*[12].

Hydrolysis of polysaccharide A with sulphuric acid, followed by chromatographic analysis of the resulting syrup showed the presence of arabinose, galactose, xylose, rhamnose and glucose, and quantitative estimation by elution on paper, and estimation with periodate[13] and by the Nelson-Symogyi[14] method, showed these sugars to be present in the relative molecular proportions of approximately 16 : 12 : 4 : 2 : 1 respectively. Furthermore, every sample of the polysaccharide isolated by the different methods of purification always gave, on hydrolysis, the same five sugars, in what appeared to be the same proportions when examined on the paper chromatogram. Partial hydrolysis with oxalic acid in an atmosphere of carbon dioxide, and separation of the hydrolysate on a cellulose column, led to the isolation of all five sugars in a crystalline form, together with a small quantity of a syrupy disaccharide. The sugars were characterised by melting point, rotation and the isolation of known crystalline derivatives. The disaccharide fraction, after purification, gave a single spot R_f 0·13, R_G 0·17 in butanol-pyridine-benzene-water (5 : 3 : 1 : 3) and, after hydrolysis, gave spots corresponding to glucose and arabinose. The possibility that this disaccharide was an artefact was disproved by the hydrolysis of an artificial mixture of chromatographically pure specimens of the five sugars under the conditions used for the hydrolysis and neutralisation of the polysaccharide. No disaccharide or any other reversion product could be detected in the solution on subsequent paper chromatography.

The possibility that polysaccharide A was more than a single entity has also been investigated. Fractional precipitation of the polysaccharide and the acetylated polysaccharide (Acetyl=20%)

gave the five sugars in the same relative intensities, on hydrolysis of each of the fractions and examination on the paper chromatogram. Dr. C. T. GREENWOOD of the Chemistry Department, Edinburgh, kindly undertook electrophoresis in a 1% solution of this polysaccharide in borate buffer pH 10. He obtained a Schlieren diagram with a symmetrical peak, indicating a monodispersed polymer.

Partial removal of the sulphate residues was achieved by heating with *N*-sodium hydroxide at 90°, in an atmosphere of nitrogen, for 8 hours. A degraded polysaccharide was isolated in 51% yield, with sulphate=13·6, and nitrogen=0·6%. Examination of the hydrolysate of this degraded material showed no obvious change in the relative proportions of the sugars present. This degraded polysaccharide gave a positive Selivanoff test for an anhydro-sugar residue on standing overnight. Similar tests on polysaccharide A and on a blank gave negative results.

After conversion to the sodium salt, periodate uptake during 36 hours was equal to 347 g polysaccharide A, for each gram molecule of periodate. Treatment for 48 hours with periodate gave an oxypolysaccharide in 85% yield, which contained the five sugars, arabinose, galactose, xylose, rhamnose and glucose in molecular proportions approximately equal to 32 : 7 : <1 : 6 : 3 respectively. Comparison with the proportions found in the original polysaccharide showed that a considerable reduction in the quantity of galactose and xylose had taken place, and that the majority of the units of these two sugars had been attacked by periodate. A pentose residue, unless it occurs at the end of a polysaccharide chain, has only two free hydroxyl groups. If these are contiguous, then the residue will be attacked by periodate, but if either of these hydroxyl groups carry a sulphate group, then the pentose will be immune to attack by periodate. It would appear, therefore, that both the galactose and the xylose residues have adjacent free hydroxyl groups, since they are attacked by periodate, and that the xylose units are either situated at the ends of chains, or are not esterified with sulphate. Assuming xylose to be free from sulphate, then calculation shows that one in every two of the remaining sugar units in polysaccharide A carry a sulphate residue.

Partial hydrolysis experiments have been carried out in an attempt to isolate and identify small portions of the polysaccharide molecule. Hydrolysis with 0·1*N*-oxalic acid at room temperature

for 5 weeks split off a small quantity of galactose only; 8 hours at 50° with the same acid in an atmosphere of carbon dioxide removed mainly galactose and xylose with a trace of arabinose and a disaccharide. 0·5*N*-Oxalic acid, at 100°, for 7 hours, under carbon dioxide gave a hydrolysate, which contained all five sugars together with the previously isolated disaccharide, and a residual degraded polysaccharide was isolated in 8–10% yield from this solution.

Simultaneous deacetylation and methylation of polysaccharide A has been carried out, and a methylated product isolated with a methoxyl content of 20%. A preliminary hydrolysis of this material indicated the presence of tetramethyl galactose.

Briefly then, the water soluble polysaccharide from Cladophora rupestris appears to be a single individual. It is a sulphated polysaccharide, and contains the five sugars, arabinose, galactose, xylose, rhamnose and glucose; the first two sugars accounting for over three-quarters of the molecule. The majority of the galactose and xylose residues are attacked by periodate and must therefore contain two contiguous unsubstituted hydroxyl groups. Partial hydrolysis experiments preferentially remove galactose and xylose residues, indicating that these two sugars are situated near the ends of the polysaccharide chains. This is further supported for galactose by the identification of tetramethyl galactose, in the hydrolysate of the methylated polysaccharide.

Until more evidence is available from the methylation of the various fragments, and from the methylated whole polysaccharide, it is impossible to reach any further decision concerning the fine structure of this polymer, which in conclusion I would suggest might be named Cladophoran.

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DISCUSSION

Dr. Young, Canada, stated that there was no question of the identity of the isolated sugar. In his experience, at least in the case of *Chondrus*, proteins are very difficult to dissolve. He asked Dr. Percival if the nitrogen might be in the form of amino acids or simple polypeptides, and if she had tried to remove the nitrogen by dialysis. Secondly, he was not convinced that the physical approach to homogeneity established it entirely since electrophoretic techniques are not quite satisfactory.

Dr. Percival answered that there might possibly be more than one polysaccharide. She had tried dialysis. Each fraction of the polysaccharide was dialysed for a week after treatment with trichloroacetic acid. She was not completely convinced from the physical evidence that the polysaccharide was homogeneous. All the methods she had tried up to the present had failed to show any separation whatsoever.

Mr. Jensen, Norway, asked Dr. Percival if she had determined the viscosity of the polysaccharide in solution, to which she answered that no viscosity measurements had been carried out.

Dr. Woodward, Scotland, was interested in the gelling properties of the polysaccharide and Dr. Percival stated that they got an increased viscosity by concentration, but the polysaccharide did not gel to the same extent as agar. She added that it had been suggested to her that the presence of anhydro sugar units had something to do with the gellifying ability but that they had failed to obtain any positive evidence for the presence of anhydro sugar units in the polysaccharide they had isolated.

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Paper Chromatography of Uronic Acids.

By R. A. Edington and Elizabeth Percival.

[Reprint Order No. 6400.]

Although paper chromatography of uronic acids has been mentioned in the literature on a number of occasions (Lederer and Lederer, " Chromatography," Elsevier, London, 1953, p. 158—171) there are remarkably few reliable records of the R_G values of the uronic acids and their methyl ethers. This is probably owing, first, to the scarcity of authentic specimens of the uronic acids, and, secondly, to the practical difficulties of " trailing " and poorly defined spots. A number of methylated uronic acids synthesised in this laboratory (Edington, Hirst, and Percival, *J.*, 1955, 2281) were, therefore, examined on the paper chromatogram, various solvent systems and spray reagents being used. Most of the chromatograms were run in a room thermostatically controlled at 21°, although comparable chromatograms run at temperatures between 18° and 21° gave the same R_G values. From preliminary experiments it appeared that for most purposes the two types of solvent system available were, on the one hand, those containing acetic acid, typified by butanol–acetic acid–water, and on the other the butanol–formic acid–water system proposed by Smith *et al.* (*J.*, 1952, 2637). The properties of both of these solvent mixtures slowly change owing to esterification of the acid by the butanol. While Smith and Spriesterbach (*Nature*, 1954, **174**, 467) have recently overcome this difficulty by coating the paper with alginic acid before elution, we have obtained consistent results by the use of an aged or otherwise equilibrated mixture. Although rhamnose has been proposed as a control substance for the paper chromatography of uronic acids, we found that it gave very variable $R_{rhamnose}$ values for the various uronic acids, whereas tetramethylglucose gave reasonably consistent results, the derived R_G values of the uronic acids being generally within 3% of one another on different papers and at different times, butanol–aqueous–formic acid being used. In the case of butanol–acetic acid–water, however, results were less consistent, the maximum variation in the R_G value of the uronic acid being about 6%. The formic acid system has certain other advantages over those containing acetic acid: it is faster, gives better separation, and is much less liable to cause badly shaped spots or trails through incomplete de-ionisation. It is noteworthy that the " heart-shaped spot " which is occasionally mentioned in the literature (Partridge, *Biochem. J.*, 1948, **42**, 238; Reid, *J. Sci. Food Agric.*, 1950, **1**, 234; Chanda, Hirst, and Percival, *J.*, 1951, 1240) as being characteristic of uronic acids has, in the present work, been observed only with solvents containing acetic acid in the presence of inorganic cations. As a rule, the spots are discrete and spherical or elliptical in outline. Aqueous aniline oxalate and butanolic *p*-anisidine hydrochloride were the most generally useful spray reagents. With uronic acids having a free hydroxyl group at $C_{(2)}$, these spray reagents give a brown or reddish-brown colour, whereas with acids substituted at $C_{(2)}$ a brilliant red or purple colour is produced.

Methyl glycosiduronic acids were generally hydrolysed, before chromatography, with N-sulphuric acid at 100° for 24 hr., followed by neutralisation with barium carbonate, filtration, and de-ionisation with Amberlite IR-100-H cation-exchange resin. Butanol–acetic acid–water (40 : 10 : 50) and butanol–formic acid–water (500 : 115 : 385) were either kept at room temperature for 14 days or boiled under reflux for 1 hr. before use.

The Table summarises the results obtained.

Uronic acid	Colour with aniline oxalate	Colour with *p*-anisidine hydrochloride	R_G in BuOH–H·CO_2H–H_2O	R_G in BuOH–AcOH–H_2O
D-Galacturonic acid	Brown	Brown	0·03	0·15
D-Mannuronic acid	Brown	Brown	0·05	0·16
D-Mannuronolactone	Brown	Brown	0·13	0·29
2-*O*-Methyl-D-galacturonic acid	Orange-red	Red-purple	0·20	0·22
D-Glucuronolactone	Brown	Brown	0·21	0·37
4-*O*-Methyl-D-mannuronic acid	Red-brown	Red-brown	0·25	0·29
3 : 4-Di-*O*-methyl-D-galacturonic acid	Red-brown	Red-brown	0·43	0·46
2 : 3-Di-*O*-methyl-D-glucuronic acid	Orange-red	Red-purple	0·47	0·56
2 : 3 : 4-Tri-*O*-methyl-D-galacturonic acid	Orange-red	Red-purple	0·63	0·61
2 : 3 : 4-Tri-*O*-methyl-D-mannuronic acid	Orange-red	Red-purple	0·79	0·80
2 : 3 : 4-Tri-*O*-methyl-D-glucuronic acid	Orange-red	Red-purple	0·84	0·84

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174. *The Synthesis of Glucosamine 6-(Dihydrogen Phosphate).*

By J. M. Anderson and Elizabeth Percival.

A crystalline monohydrate of glucosamine 6-(dihydrogen phosphate) has been synthesised from glucosamine *via* 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine. All the intermediate derivatives prepared were crystalline and were characterised. Periodate oxidation confirmed the structure of the 6-phosphate.

Brown [1] records the phosphorylation of glucosamine by hexokinase and the isolation of a small quantity of glucosamine 6-phosphate. He considers that the process may be important in the series of anabolic stages whereby naturally occurring polysaccharides containing glucosamine residues are produced. More recently Leloir and Cardini [2] isolated enzymes from the mould, *Neurospora crassa*, which catalyse the change of hexose 6-phosphate into glucosamine 6-phosphate in the presence of glutamine and of acetylglucosamine 1-phosphate into the corresponding 6-phosphate. This led Kalckar and Klenow [3] to suggest that " the formation of glucosamine 6-phosphate should also be considered as a pathway in the biosynthesis of purine ribosides, the more so since the nitrogen of the *N*-ribosidic linkage in purine ribosides is derived specifically from the amide nitrogen of glutamine." In view of the wide interest in this derivative of glucosamine it appeared to us that an authentic synthesis, whereby supplies of this derivative might be available for more extensive enzymic studies, was desirable.

Direct phosphorylation of glucosamine led to an impure product.[4] In order to prepare glucosamine 6-phosphate from glucosamine it is necessary first to protect the secondary hydroxyl groups and the amino-group with other residues. Preliminary experiments were carried out on crystalline *N*-*p*-methoxybenzylidene-D-glucosamine. It was hoped to prepare 1 : 3 : 4-tri-*O*-acetyl-*N*-*p*-methoxybenzylidene-6-*O*-triphenylmethyl-D-glucosamine and, after removal of the triphenylmethyl residue, to phosphorylate the product. Unfortunately attempts to prepare crystalline derivatives were unsuccessful and these experiments were abandoned. The conditions necessary for the hydrolysis of *O*-acetyl and *N*-acetyl groups in glucosamine were studied, and, as these did not appear to be likely to cause the removal of a 6-phosphate group, a synthesis was begun with 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-6-*O*-triphenylmethyl-D-glucosamine as intermediate.

Crystalline *N*-acetyl-D-glucosamine was prepared, and after tritylation and acetylation 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-6-*O*-triphenylmethyl-β-D-glucosamine with carbon tetrachloride of crystallisation was isolated in 25% yield. From the mother-liquors 5·5% of the α-anomer with mixed solvent of crystallisation was obtained. Further treatment of the mother-liquors gave 2% of 3 : 4-di-*O*-acetyl-*N*-acetyl-1 : 6-di-*O*-triphenylmethyl-D-glucosamine. The phenomenon of solvent of crystallisation is seldom encountered in non-ionic sugar derivatives. Triphenylmethyl ethers, however, appear to be more liable than most derivatives to behave in this way. For example, the preparation of an ethanol solvate of triphenylmethyldulcitol has been reported [5] and Edington, Hirst, and Percival [6] isolated methyl tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-mannopyranoside with various solvents of crystallisation.

When acetic acid at 100° was used to remove the triphenylmethyl residues from the tetra-acetates [7] hydrolysis of the 1-acetyl group also occurred, but by treatment of the β-acetate with a cold saturated solution of hydrogen bromide in glacial acetic acid for not longer than 60 seconds crystalline 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine was obtained in 67% yield. This product could not be isolated by the method used for 1 : 2 : 3 : 4-tetra-acetyl-β-D-glucose.[8] The three times greater solubility of the tetra-acetyl-β-D-glucosamine in water than in chloroform made it necessary to employ exhaustive chloroform extraction and to eliminate the usual water washing of the extract.

In view of the larger yield of the β-anomer of the triphenylmethyl ether, and the fact that all attempts to isolate crystalline 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-α-D-glucosamine were unsuccessful the synthesis was continued in the β-series. As the possibility of acetyl migration [9] during the removal of the triphenylmethyl group could not be ignored the structure of the 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine was confirmed by reconversion into the 6-triphenylmethyl ether, and by toluene-*p*-sulphonylation followed by the replacement of the toluene-*p*-sulphonyl group by iodine under the standard conditions for the replacement of a primary toluene-*p*-sulphonyloxy-group.[10]

Phosphorylation of the tetra-acetyl-β-D-glucosamine was carried out with diphenyl phosphorochloridate and crystalline 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine 6-(diphenyl phosphate) was isolated in 70% yield. Hydrogenolysis of the phenyl groups gave a good yield of the crystalline 6-(dihydrogen phosphate). The acetyl groups were hydrolysed with sulphuric acid and a barium salt was precipitated by ethanol after neutralisation of the hydrolysate with barium carbonate. The difficulty of converting all the phosphate into the barium salt combined with that of removing all the inorganic barium salts made the isolation of a pure salt difficult. Exact neutralisation of the sulphuric acid hydrolysate with barium hydroxide led to the crystallisation of the monohydrate of D-glucosamine 6-(dihydrogen phosphate). In addition a small yield of a crystalline dihydrate was isolated on autohydrolysis of the tetra-acetyl phosphoric acid derivative. Chromatographic analysis of the free acid and of the barium salt (after removal of barium) showed identical spots.

The constitution of the dihydrogen phosphate and of the barium salt was confirmed by periodate oxidation :[1] both took up the theoretical quantity of periodate (Table 1) and gave no formaldehyde (Table 2) :

TABLE 1. *Uptake of periodate in moles/mole of sugar.*

Time (hr.)	22	64	94	Theor.
Glucosamine hydrochloride	4·8	4·9	4·9	5
Glucosamine 6-(barium phosphate)	3·8	3·9	3·9	4
Glucosamine 6-(dihydrogen phosphate)	3·9	3·9	3·9	4

TABLE 2. *Formaldehyde release.*

	Mole of CH_2O/mole of sugar	Theor.
Glucosamine hydrochloride	0·81	1·0
N-Acetyl-α-D-glucosamine	0·98	1·0
Glucosamine 6-(barium phosphate)	0·00	0·0
Glucosamine 6-(dihydrogen phosphate)	0·00	0·0

Estimation of the glucosamine content of the 6-phosphate by the Elson–Morgan technique gave 98% of glucosamine.

The rate of hydrolysis of the phosphate group was compared with that of glucose 6-phosphate.[11] Whilst glucosamine 6-phosphate was 50% hydrolysed during 73 hours, glucose 6-phosphate had undergone 50% hydrolysis in 23 hours under the same conditions.

EXPERIMENTAL

Solvents were removed under reduced pressure below 50°. M. p.s were determined on the Kofler hot-stage microscope. Optical rotations were determined at 20° ± 2° in $CHCl_3$ unless otherwise stated. Light petroleum was the fraction of b. p. 60—80°.

1 : 3 : 4-*Tri*-O-*acetyl*-N-*acetyl*-6-O-*triphenylmethyl*-D-*glucosamine*.—D-Glucosamine hydrochloride (130 g.) was converted into crystalline *N*-acetyl-α-D-glucosamine (115 g., 96%) by the method of Roseman and Ludowieg.[12] After recrystallisation from aqueous ethanol–ether the crystals had m. p. 209—210° (decomp.), $[\alpha]_D^{18}$ +41° (Found : C, 43·4; H, 6·5; N, 6·9. Calc. for $C_8H_{15}O_6N$: C, 43·4; H, 6·8; N, 6·3%).

N-Acetyl-α-D-glucosamine (75 g.) in dry pyridine (500 ml.) in the presence of " Drierite " (8 g.) was heated with triphenylmethyl chloride (98 g.) at 100° until the reactants had dissolved (20 min.).[13] Acetic anhydride (150 ml.) was added to the hot solution and, after cooling overnight, the mixture was filtered and the excess of triphenylmethyl chloride in the filtrate decomposed with ice-water. The product was isolated as a white amorphous solid by pouring

the mixture into vigorously stirred ice-water (3000 ml.). After drying, the product (170 g., 85%) had $[\alpha]_D$ +32° (*c*, 1·1). Solution of the crude product in hot carbon tetrachloride gave, on cooling, colourless needles (68 g.) of the carbon tetrachloride *solvate* of 1 : 3 : 4-*tri*-O-*acetyl*-N-*acetyl*-6-O-*triphenylmethyl*-β-D-*glucosamine*. These crystals lost solvent at 100—110° and had m. p. 187—189°, $[\alpha]_D$ +22° (*c*, 1·4) (Found : C, 52·3; H, 4·7; N, 2·3; Cl, 21·9; loss at 100°/15 mm. in 18 hr., 26·2. $3C_{33}H_{35}O_9N,4CCl_4$ requires C, 51·9; H, 4·4; N, 1·8; Cl, 23·8; CCl_4, 25·8%). The *amine* freed from solvent had m. p. 189—191°, $[\alpha]_D$ +33° (*c*, 1·0) (Found : C, 66·8; H, 5·7; N, 2·0. $C_{33}H_{35}O_9N$ requires C, 67·2; H, 6·0; N, 2·4%). When an ethanolic solution of the crystals was heated with Fehling's solution for several minutes a positive reducing test was obtained.

After removal of the β-anomer, evaporation of the carbon tetrachloride from the mother-liquors gave a syrup, which, on dissolution in warm chloroform and treatment with an equal volume of ether, gave crystals (11·0 g.) of a mixed solvate of 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-6-*O*-triphenylmethyl-α-D-glucosamine, m. p. 111—112°, $[\alpha]_D$ +90° (*c*, 0·9) (Found : C, 66·6; H, 6·0; N, 2·6; Cl, 2·7; loss at 100°/15 mm. in 20 hr., 5·6%). Freed from solvent the *amine* had m. p. 154—156°, $[\alpha]_D$ +97° (*c*, 1·2) (Found : C, 66·9; H, 6·0; N, 1·8%). These crystals also reduced Fehling's solution if tested as above.

The residual mother-liquors, when warmed and treated with light petroleum to turbidity, deposited, on cooling, crystals of 3 : 4-*di*-O-*acetyl*-N-*acetyl*-1 : 6-*di*-O-*triphenylmethyl*-D-*glucosamine* (4 g.), m. p. 254—256°, $[\alpha]_D$ −29° (*c*, 1·0), non-reducing to Fehling's solution (Found : C, 75·9; H, 6·0; N, 2·2. $C_{50}H_{49}O_8N$ requires C, 76·0; H, 6·0; N, 1·8%).

3 : 4-*Di*-O-*acetyl*-N-*acetyl*-α-D-*glucosamine*.—To the bistriphenylmethyl ether (1·01 g.) in glacial acetic acid (18 ml.) at 100°, water (12 ml.) was added dropwise and the temperature kept at 100° for 60 min. Cooling, addition of water (60 ml.), filtration, and concentration gave a syrup (390 mg.) which on trituration with chloroform partially crystallised. This 3 : 4-*di*-O-*acetyl*-N-*acetyl*-α-D-*glucosamine* (150 mg., 38%), after recrystallisation from acetone–light petroleum, gave a negative test for the triphenylmethyl group [14] and had m. p. 186—187°, $[\alpha]_D$ +75° (*c*, 0·9), $[\alpha]_D^{18}$ +65° (5 min., *c*, 1·0 in H_2O) → +35° (8 hr., const.) (Found : C, 47·2; H, 6·3; N, 4·5. $C_{12}H_{19}O_8N$ requires C, 47·2; H, 6·3; N, 4·6%).

1 : 3 : 4-*Tri*-O-*acetyl*-N-*acetyl*-β-D-*glucosamine*.—In the following experiments the carbon tetrachloride solvate of the triphenylmethyl ether was used.

(*a*) Treatment as in the previous experiment with glacial acetic acid (36 ml.) of 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-6-*O*-triphenylmethyl-β-D-glucosamine (2·0 g.) gave a syrup (800 mg.), whose cooled solution in ethanol gave crystals of 3 : 4-di-*O*-acetyl-*N*-acetyl-α-D-glucosamine (330 mg., 43%), which on recrystallisation from ethanol had m. p. and mixed m. p. 188—189°, $[\alpha]_D^{19}$ +68° (5 min., *c*, 1·0 in H_2O) → +32° (8 hr., const.) (Found : C, 47·1; H, 6·2; N, 4·7%).

(*b*) A cooled solution of the β-monotriphenylmethyl ether (10 g.) in acetic acid (32 ml.) was shaken with a cooled saturated solution of hydrogen bromide in acetic acid (3·2 ml.) with cooling for 60 sec. The precipitated triphenylmethyl bromide was removed and the filtrate poured into ice-water (200 ml.). This liquid mixture and the washings (300 ml.) from the bromide were extracted with chloroform (8 × 300 ml.). The extracts were dried without washing and on concentration gave a syrup. Residual chloroform and acetic acid were removed by repeated distillation with dry toluene. A crystalline residue (2·9 g., 67%), m. p. 169—171°, was obtained. After two recrystallisations from chloroform–ether, these crystals of 1 : 3 : 4-*tri*-O-*acetyl*-N-*acetyl*-β-D-*glucosamine* had m. p. 175—176°, $[\alpha]_D^{18}$ +5·5° (*c*, 1·6), $[\alpha]_D^{18}$ +18° (*c*, 1·1 in H_2O) (Found : C, 48·1; H, 6·2; N, 4·0. $C_{14}H_{21}O_9N$ requires C, 48·4; H, 6·1; N, 4·0%).

Triphenylmethylation of 1 : 3 : 4-*Tri*-O-*acetyl*-N-*acetyl*-β-D-*glucosamine*.—The foregoing β-tetra-acetate (200 mg.) and triphenylmethyl chloride (160 mg., 1 mol.) in pyridine (2 ml.) at 50° (60 hr.) in the presence of " Drierite " gave, after the usual treatment, the carbon tetrachloride solvate of 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-6-*O*-triphenylmethyl-β-D-glucosamine as needles (150 mg., 33%), $[\alpha]_D$ +24° (*c*, 1·2). The crystals lost solvent at 100—110° and the desolvated material had m. p. and mixed m. p. with the specimen previously prepared 185—187°.

1 : 3 : 4-*Tri*-O-*acetyl*-N-*acetyl*-6-O-*toluene*-p-*sulphonyl*-β-D-*glucosamine*.—A solution of 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine (100 mg.) and toluene-*p*-sulphonyl chloride (110 mg., 2 mol.) in pyridine (1 ml.) in the presence of " Drierite " at room temperature (72 hr.) gave a syrup (105 mg.) which crystallised under methanol. 1 : 3 : 4-*Tri*-O-*acetyl*-N-*acetyl*-6-O-*toluene*-p-*sulphonyl*-β-D-*glucosamine* had m. p. 170—171°, $[\alpha]_D$ +16° (*c*, 1·1) (Found : C, 50·7; H, 5·4; S, 6·1. $C_{21}H_{27}O_{11}NS$ requires C, 50·3; H, 5·4; S, 6·4%).

This material (10 mg.) was heated in a sealed tube with sodium iodide (10 mg.) in acetone

at 100° for 2 hr.[10] The mixture deposited characteristic plate-shaped crystals of sodium toluene-*p*-sulphonate.

1 : 3 : 4-*Tri*-O-*acetyl*-N-*acetyl*-β-D-*glucosamine* 6-(*Diphenyl Phosphate*).—1 : 3 : 4-Tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine (11 g.) in dry pyridine (45 ml.) in the presence of " Drierite " at 0° was treated with diphenyl phosphorochloridate[15] (9·35 g., 1·1 mol.) with shaking and cooling during 20 min. The mixture was kept at 0° for a further 15 min.[16] and, after being kept overnight at 10°, was cooled to 0° and filtered. Excess of acid chloride was decomposed by a few drops of ice-water and the mixture kept at room temperature for 30 min. Slow pouring into ice-water with stirring gave a gel which quickly changed to a white crystalline solid. This on recrystallisation from aqueous acetone gave 1 : 3 : 4-*tri*-O-*acetyl*-N-*acetyl*-β-D-*glucosamine* 6-(*diphenyl phosphate*) (11·8 g.), m. p. 144—145°, $[\alpha]_D$ +25° (*c*, 0·7) (Found : C, 53·6; H, 5·3; N, 2·7; P, 5·4. $C_{26}H_{30}O_{12}NP$ requires C, 53·9; H, 5·2; N, 2·4; P, 5·3%). Extraction of the aqueous filtrate with chloroform (2 × 250 ml.) and thorough washing of the extracts yielded on removal of the chloroform a syrup. On trituration with aqueous acetone this gave a further crop of the same crystals (1·1 g.).

1 : 3 : 4-*Tri*-O-*acetyl*-N-*acetyl*-β-D-*glucosamine* 6-(*Dihydrogen Phosphate*).—The diphenyl ester (12·6 g.) (in 3 experiments of 4·2 g. each) in aldehyde-free ethanol (total, 100 ml.) was refluxed for 25 min. with activated charcoal (3·0 g.). The charcoal was removed and the solution added to ethanol (10 ml.) containing the platinum catalyst (from 0·33 g. of Adams platinum oxide reduced *in situ* for each experiment). The solution was shaken in hydrogen at slightly >1 atm. Uptake stopped at 7·6 mol. (95%) in 18, 19, and 7 hr. severally for the 3 experiments. Removal of the solvent at 25° gave a syrup which on trituration with ethanol deposited crystals (7·09 g., 75%) of 1 : 3 : 4-*tri*-O-*acetyl*-N-*acetyl*-β-D-*glucosamine* 6-(*dihydrogen phosphate*), m. p. 166—168° (decomp.), $[\alpha]_D^{19}$ +25° (*c*, 1·0 in H_2O) (Found : C, 38·9; H, 5·0; N, 3·6; P, 7·0. $C_{14}H_{22}O_{12}NP$ requires C, 39·3; H, 5·2; N, 3·3; P, 7·3%).

D-*Glucosamine* 6-(*Dihydrogen Phosphate*).—(a) *Barium salt.* 1 : 3 : 4-Tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (400 mg.) was hydrolysed with N-sulphuric acid (12 ml.) at 100° until the rotation was constant (2·5 hr.) {$[\alpha]_D^{30}$ +60° (*c*, 2·0 in N-H_2SO_4) for the dipolar ion of the product}. The solution was shaken overnight with finely divided barium carbonate, then filtered, and the barium salts were washed with water. The combined filtrates were treated with ethanol (4 vol.). A white powder was precipitated which was washed with 90% aqueous ethanol, ethanol–ether (3 : 1, then 1 : 3), and ether. The dried precipitate (290 mg.; P, 6·3%) was treated in 0·01N-hydrochloric acid (6 ml.) with ethanol (24 ml.). The precipitate (150 mg., 41%) had $[\alpha]_D^{18}$ (dipolar ion) +53° (*c*, 0·3 in H_2O; pH 2·5). {Brown[17] records $[\alpha]_D^{24}$ +48·5° (*c*, 0·5 in H_2O; pH 2·5)} (Found : C, 19·6; H, 4·4; N, 4·7; total P, 7·8; inorg. P, 0·05. Calc. for $C_6H_{12}O_8NPBa$: C, 18·3; H, 3·1; N, 3·6; total P, 7·9%).

(b) *Free acid.* (i) 1 : 3 : 4-Tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (2·8 g.) was hydrolysed with N-sulphuric acid as before. The solution was neutralised with a half-saturated solution of barium hydroxide, samples (1 drop) being tested for barium with a solution of rhodizonic acid[18] in water (when all the sulphuric acid had been precipitated a red-brown precipitate of barium rhodizonate was formed). After removal of the barium sulphate by centrifugation the clear solution (pH 4) was concentrated to 50 ml. Addition of ethanol to turbidity gave crystals of the *acid* (0·81 g., 45%) after several days at 0°. This, after drying at 18°/0·1 mm., had m. p. 170—180° (decomp.), $[\alpha]_D$ +54° (*c*, 0·5 of the hydrate in H_2O) (Calc. for the anhyd. compound, +58°) (Found : C, 25·8; H, 5·7; N, 5·2; total P, 11·3; inorg. P. 0·0. $C_6H_{14}O_8NP,H_2O$ requires C, 26·0; H, 5·8; N, 5·1; P, 11·2%).

(ii) A solution of 1 : 3 : 4-tri-*O*-acetyl-*N*-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (200 mg.) in water (3 ml.) was heated at 100° for 30 hr. Decolorisation with charcoal and concentration gave a glass which on dissolution in water (4 ml.), addition of alcohol, and nucleation with the crystalline monohydrate of D-glucosamine 6-(dihydrogen phosphate) gave crystals (17 mg.) of a dihydrate. These had m. p. 165—175° (decomp.) (Found : C, 24·4; H 6·0; N, 4·6; P, 10·4. $C_6H_{14}O_8NP,2H_2O$ requires C, 24·4; H, 6·1; N, 4·7; P, 10·5%).

Chromatography by the ascending technique[19] with ethyl acetate–pyridine–formamide (6 : 1 : 3) as eluate and ferric chloride–sulphosalicylic acid spray,[20] gave a single identical spot for the glucosamine 6-(dihydrogen phosphate) applied in dilute sulphuric acid solution and the barium salt (freed from barium with sulphuric acid).

Oxidation by Periodate.—(a) *Periodate uptake.* Oxidations were carried out on glucosamine 6-(barium phosphate) and 6-(dihydrogen phosphate) and glucosamine hydrochloride.[1] The weighed substance (100—200 μ moles) was dissolved in water, the solution adjusted to pH 4·5, and the volume made up to 25 ml. A sample (6 ml.) was treated with 3 ml. of 0·1M-sodium

metaperiodate, made up to 10 ml. with distilled water, and set aside in the dark at room temperature. At suitable intervals, portions (3 ml.) were treated with sodium hydrogen carbonate (0·6 g.), potassium iodide (0·6 g.), and sodium arsenite (0·1N; 5 ml.). After 10 min. the mixture was titrated with 0·05N-iodine. Barium was removed from the barium salt by the addition of sulphuric acid (0·1N; 3 ml.) before adjustment of the pH to 4·5.

(b) *Formaldehyde release.* Solutions of the sugar derivatives (0·7 μ moles/ml.) were oxidised in buffered solution (pH 7·5, sodium carbonate) with periodate for 21 hr. and the formaldehyde released was estimated under the conditions advocated by O'Dea and Gibbons.[21] Three glucose solutions of different concentrations were used as standards, and the filter used was the Ilford spectrum yellow (606).

Elson–Morgan Estimation of Glucosamine Content.—The glucosamine content of the 6-phosphate was estimated according to the method of Elson and Morgan [22] as modified by Belcher, Nutten, and Sambrook.[23] Glucosamine solutions were used as standards, and measurements made with the Ilford spectrum green (604) filter. The crystalline monohydrate of glucosamine 6-(dihydrogen phosphate) was found to have 98% of its theoretical glucosamine content. The optical densities of the coloured solutions from glucosamine hydrochloride and the 6-(di hydrogen phosphate) (each 0·05 μg. of glucosamine per ml.) were examined at 450—600 mμ with a Unicam SP600 spectrophotometer. It was found necessary to use 2-cm. cells to obtain optical densities equivalent to those Belcher and his co-workers [23] describe for 2-mm. cells, and, although the colours from glucosamine and the 6-phosphate appeared identical to the eye, when the optical densities were plotted against the wavelength there was a slight displacement (512 to 518 mμ) in the position of the maximum together with a minor difference in the shape of the curve. This may be due to the production of two similar chromophores in each case, but in rather different quantities.

Hydrolysis of D-*Glucosamine* 6-(*Dihydrogen Phosphate*).—The crystalline monohydrate of glucosamine 6-(dihydrogen phosphate) (67·9 mg.) in N-hydrochloric acid (25 ml.) was heated at 100° and aliquot parts were analysed at suitable times for hydrolysed phosphorus. Robison [11] carried out an identical hydrolysis of glucose 6-phosphate and his figures are given in the Table for comparison.

Time (hr.)	0	4	8	20	45	70	80
Phosphorus (mg./ml.)	0	0·013	0·021	0·047	0·104	—	0·160
Hydrolysis (%) :							
Glucosamine 6-dihydrogen phosphate	0	4·3	6·8	15·6	34·2	—	52·6
Glucose 6-phosphate [11]	—	12·7	21·6	44·9	75·0	88·9	—

Hydrolysis of 1 : 3 : 4 : 6-*Tetra*-O-*acetyl*-N-*acetyl- and* N-*Acetyl-glucosamine.*—4% Solutions were hydrolysed with N-hydrochloric acid at 100° and the hydrolyses followed polarimetrically. (*a*) Tetra-*O*-acetyl-*N*-acetyl-β-D-glucosamine: α_D +1·99° (2 min); +1·36° (42 min.); +1·43° (57 mins.); +1·43° (70 min.). Crystalline D-glucosamine hydrochloride (84%), $[\alpha]_D^{18}$ +86° (3 min.) ⟶ +70° (const.) (*c*, 1·5 in H_2O), was recovered from the hydrolysate. (*b*) *N*-Acetyl-α-D-glucosamine: α_D +1·42° (initial); +2·65 (2 hr.); +2·65° (3 hr.).

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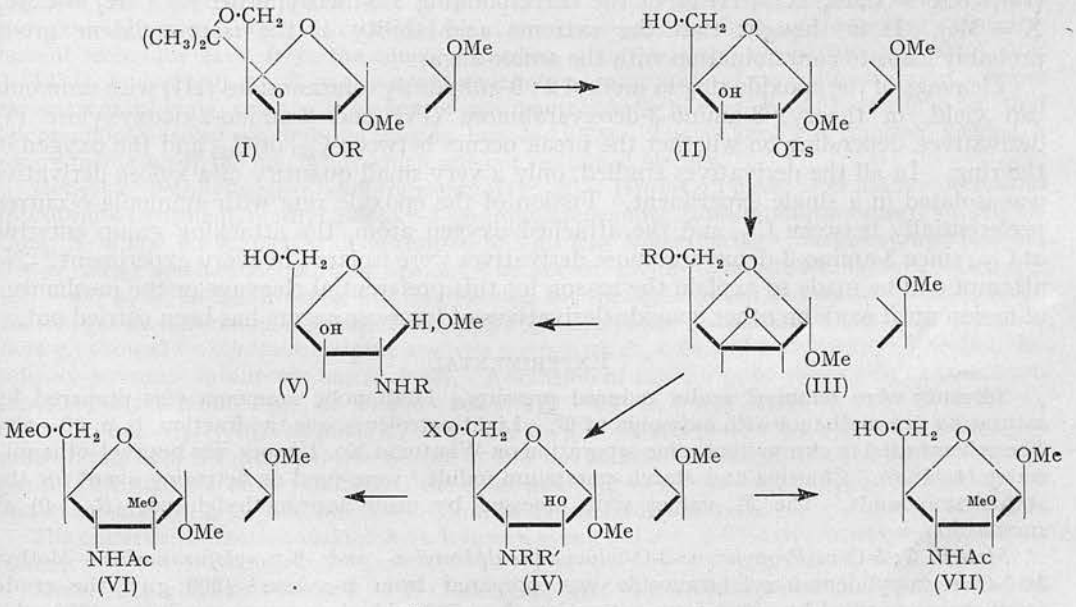
175. *The Ammonolysis of Methyl 2 : 3-Anhydro-D-furanosides. Part II.* Methyl 2 : 3-Anhydro-5-O-methyl-α- and -β-D-lyxofuranosides.*

By J. M. Anderson and Elizabeth Percival.

Ammonolyses of methyl 2 : 3-anhydro-5-*O*-methyl-α- and -β-D-lyxofuranosides led to the isolation of crystalline derivatives of methyl 3-amino-3-deoxy-5-*O*-methyl-α- and -β-D-arabinosides. During this work methyl 2-acetamido-2-deoxy-β-D-xylofuranoside, 3-acetamido-3-deoxy-2-*O*-methyl-β-D-arabofuranoside, and 3-acetamido-3-deoxy-2 : 5-*O*-dimethyl-α- and -β-D-arabinosides and two new derivatives of methyl D-xylofuranoside have been prepared.

Percival and Zobrist [1] reported the synthesis of methyl 2 : 3-anhydro-α-D-lyxofuranoside (III; R = H) from a mixture of methyl 3 : 5-*O*-*iso*propylidene-α- and -β-D-xylofuranosides (I; R = H) by tosylation (I; R = Ts), mild acid hydrolysis (II), and alkaline hydrolysis of the tosyl residue. Subsequently Baker, Schaub, and Williams,[2] from a similar synthesis, isolated the β-anomer of the 2 : 3-anhydro-sugar (III; R = H), after first partly separating methyl 3 : 5-*O*-*iso*propylidene-αβ-D-xylofuranoside into its two anomers by distillation. Although these authors carried out the subsequent syntheses separately on the two fractions and isolated crystalline methyl 2 : 3-anhydro-α- and -β-D-lyxofuranosides, none of their intermediate derivatives was obtained crystalline. During the present work fractional distillation of the *iso*propylidene-αβ-xylofuranoside led to the separation of two fractions, whose specific rotations differed considerably from the values recorded by Baker and his co-workers. That the present separation was more efficient was confirmed by the ready isolation of the two crystalline methyl 3 : 5-*O*-*iso*propylidene-2-*O*-toluene-*p*-sulphonyl-D-xylofuranosides (I; R = Ts). Hydrolysis of the *iso*propylidene group with boiling 1% methanolic hydrogen chloride [3] or at 50° with 80% acetic acid [2] caused anomerisation; by use of 0·1% methanolic hydrogen chloride at room temperature, however, no anomerisation occurred and crystalline methyl 2-*O*-toluene-*p*-sulphonyl-α-D-xylofuranoside (II) was obtained in excellent yield.



Crystalline methyl 2 : 3-anhydro-α- [1] and -β-lyxofuranosides [2] were prepared and, after ammonolysis followed by treatment with acetone, gave methyl 3-deoxy-3-*iso*propylidene-

* Part I, Anderson and Percival, *J.*, 1955, 1042.

amino-α- and -β-D-arabofuranosides (IV; RR′ = CMe_2, X = H), respectively. The mother liquors were examined for methyl 2-amino-2-deoxyxylofuranoside (V; R = H) derivatives. The *N*-acetyl-3 : 5-*iso*propylidene derivative appeared to be the most appropriate to prepare, but specific *N*-acetylation and attempted condensation with acetone failed to yield any crystalline derivatives from the mother liquors of the α-anomer. The mother liquors of the β-anomer, however, gave crystals (0·6% of the ammonolysis product) which gave correct analytical results for a methyl 2-acetamido-2-deoxyxylofuranoside (V; R = Ac) and were converted into a free sugar hydrochloride whose rotation was identical with that recorded [4] for 2-amino-2-deoxy-D-xylose hydrochloride.

Methylation of both anomers of methyl 3-acetamido-3-deoxy-D-arabofuranoside [5] (IV; R = H, R′ = Ac, X = H) gave the respective crystalline 2 : 5-dimethyl ethers (VI). Complete methylation proved difficult in the β-series; after three treatments two crops of crystals were obtained. The first was shown by chromatography to be a single entity and by analysis to be a methyl 3-acetamido-3-deoxymonomethylarabinoside. Determination of melting point of mixtures showed that it was not the 5-methyl ether, and conversion into a toluene-*p*-sulphonate which showed the presence of a primary toluene-*p*-sulphonyloxy-group [6] proved it to be methyl 3-acetamido-3-deoxy-2-*O*-methyl-β-D-arabofuranoside (VII). The second crop of crystals was shown by paper chromatography and by analysis to be a mixture of mono- and di-methyl ethers. Three further methylations of the mother liquors were necessary before the pure dimethyl ether (VI) could be isolated.

Methylation of both anomers of methyl 2 : 3-anhydro-D-lyxofuranoside (III; R = H) gave crystalline α- and β-5-methyl ethers (III; R = Me) which on ammonolysis and appropriate treatment gave crystalline *N-iso*propylidene and *N*-acetyl derivatives. That these were derivatives of 3-amino-3-deoxy-D-arabinose was proved by the isolation, after complete methylation, of crystalline dimethyl ethers identical with the 2 : 5-dimethyl ethers (VI) obtained above. No crystalline derivatives of xylose could be isolated from the mother liquors after the removal of the *N-iso*propylidene (α-5-methyl) and *N*-acetyl (β-5-methyl) arabinose derivatives.

Although Baker and his co-workers [2] record sharp melting points and correct analyses for the *N-iso*propylidene derivatives, we were unable, even after repeated recrystallisation, to isolate crystals of pure methyl 3-deoxy-3-*iso*propylideneamino-α- or -β-arabofuranosides (IV; RR′ = CMe_2, X = H) or of the corresponding α-5-methyl ether (IV; R_2 = CMe_2, X = Me). It is thought that the extreme acid-lability of the *iso*propylidene group probably leads to contamination with the amino-sugar.

Cleavage of the epoxide ring in methyl 2 : 3-anhydrolyxofuranosides (III) with ammonia can yield, in theory, 3-amino-3-deoxyarabinose (IV) and 2-amino-2-deoxyxylose (V) derivatives, depending on whether the break occurs between $C_{(3)}$ or $C_{(2)}$ and the oxygen of the ring. In all the derivatives studied, only a very small quantity of a xylose derivative was isolated in a single experiment. Fission of the epoxide ring with ammonia occurred preferentially between $C_{(3)}$ and the attached oxygen atom, the attacking group entering at $C_{(3)}$, since 3-amino-3-deoxyarabinose derivatives were isolated in every experiment. No attempt will be made to explain the reason for this preferential cleavage or the mechanism of fission until work on other epoxide derivatives of furanose sugars has been carried out.

Experimental

Solvents were removed under reduced pressure. Methanolic ammonia was prepared by saturating dry methanol with ammonia at 0°. Light petroleum was the fraction, b. p. 60—80°. The solvent used in chromatographic separation on Whatman No. 1 paper was butanol–ethanol–water (4 : 1 : 5). Chlorine and starch–potassium iodide [7] were used as detecting agent for the amino-compounds. The R_G values were assessed by using tetramethylglucose (R_G 1·0) as standard.

Methyl 3 : 5-O-iso*Propylidene*-2-O-*toluene*-p-*sulphonyl*-α- *and* -β-D-*xylofuranoside*.—Methyl 3 : 5-*O-iso*propylidene-D-xylofuranoside was prepared from D-xylose [1] (200 g.); the crude product was purified by chloroform extraction (3 × 200 ml.) of an aqueous solution (200 ml.). Repeated fractional distillation (fractions of similar specific rotation were combined and refractionated) of the concentrated chloroform extracts (172 g.; in 30 g. portions) through a Vigreux column led finally to the separation of two fractions. Fraction I: 71·6 g. (42%), b. p.

60—70°/0·05 mm., $[\alpha]_D$ +75° (*c*, 1·0 in $CHCl_3$; average: range, +70°—+82°), n_D^{18} 1·4683. Fraction II: 55·2 g. (32%), b. p. 90—100°/0·05 mm., $[\alpha]_D$ −80° (*c*, 1·0 in $CHCl_3$; average: range, −65° to −85°), n_D^{17} 1·4650. Baker *et al.*[2] record for fraction I, $[\alpha]_D^{24}$ +18° in H_2O, and for fraction II, $[\alpha]_D^{24}$ −64° in H_2O.

Methyl 3 : 5-*O*-*iso*propylidene-D-xylofuranoside (71 g.; $[\alpha]_D$ +75°) was treated in pyridine (120 ml.) with toluene-*p*-sulphonyl chloride (73 g.).[8] A crystalline product (92 g., 74%), m. p. 77°, $[\alpha]_D^{21}$ +66° (*c*, 1·4 in $CHCl_3$), was isolated. After recrystallisation from light petroleum, *methyl* 3 : 5-O-iso*propylidene*-2-O-*toluene*-p-*sulphonyl*-α-D-*xylofuranoside* (I; R = Ts) had m. p. 79—80°, $[\alpha]_D^{18}$ +68° (*c*, 1·1 in $CHCl_3$) (Found: C, 53·2; H, 6·1; S, 8·9. $C_{16}H_{22}O_7S$ requires C, 53·2; H, 6·2; S, 9·0%).

Methyl 3 : 5-*O*-*iso*propylidene-D-xylofuranoside (55 g.; $[\alpha]_D$ −80°) after similar treatment gave a product (80 g.), $[\alpha]_D^{18}$ −37° (*c*, 1·1 in $CHCl_3$). Recrystallisation from aqueous methanol gave methyl 3 : 5-*O*-*iso*propylidene-2-*O*-toluene-*p*-sulphonyl-β-D-xylofuranoside (I; R = Ts) (62 g., 64%), m. p. 119—120°, $[\alpha]_D^{17}$ −53° (*c*, 0·9 in $CHCl_3$), $[\alpha]_D^{18}$ −44° (*c*, 0·4 in MeOH). This was identical with the material isolated by Percival and Zobrist [3] from syrupy methyl 3 : 5-*O*-*iso*propylidene-αβ-D-xylofuranoside.

Methyl 2-O-*Toluene*-p-*sulphonyl*-α- *and* -β-D-*xylofuranosides*.—Methyl 3 : 5-*O*-*iso*propylidene-2-*O*-toluene-*p*-sulphonyl-α-D-xylofuranoside (89 g.), dissolved in 0·1% methanolic hydrogen chloride (20 ml.), was kept at room temperature until the rotation reached a constant value: α_D +1·46° (initial), +1·95° (5 min.), +2·12 (15 min.), +2·13 (20 min.; const.). After neutralisation with silver carbonate, a syrup (75 g., 95%) was obtained which crystallised completely. Recrystallisation from chloroform–light petroleum gave *methyl* 2-O-*toluene*-p-*sulphonyl*-α-D-*xylofuranoside* (II), m. p. 90—91°, $[\alpha]_D^{25}$ +101° (*c*, 1·3 in $CHCl_3$) (Found: C, 49·5; H, 5·8; S, 10·4. $C_{13}H_{18}O_7S$ requires C, 49·1; H, 5·7; S, 10·1%). The β-form was isolated as a syrup (95%), $[\alpha]_D^{22}$ −20° (*c*, 1·0 in $CHCl_3$), n_D^{22} 1·5243.

Attempted Isolation of Xylose Derivatives.—Ammonolysis of methyl 2 : 3-anhydro-α-D-lyxofuranoside (III; R = H) (13·2 g.) by heating it with methanolic ammonia in an autoclave at 120° for 48 hr. gave, after treatment with charcoal, a brown syrup (14·6 g., 99%). This was dissolved in hot acetone (250 ml.);[2] the cooled solution deposited methyl 3-deoxy-3-*iso*propylideneamino-α-D-arabofuranoside (IV; R_2 = CMe_2, X = H). The total yield, after several treatments of the mother liquors, was 15·5 g. (85%). *N*-Acetylation of the residual material (1·8 g., 10%) in water (50 ml.) with methanol (5 ml.), resin (50 ml.), and acetic anhydride (1·25 ml.) [9] gave a syrup (1·5 g., 83%). When shaken with acetone (50 ml.), anhydrous copper sulphate (8 g.), and N-sulphuric acid (0·08 ml.),[2] and then treated appropriately, this yielded a non-reducing syrup (1·0 g.). Fractionation of this between chloroform and water by a counter-current technique gave, from the aqueous fraction, a non-reducing syrup (900 mg., 90%), $[\alpha]_D^{22}$ +112° (*c*, 1·1 in H_2O), which gave a negative iodoform test for the *iso*propylidene group. Paper chromatography showed the presence of two components, having R_G 0·67 and 0·76 (methyl 3-acetamido-3-deoxy-α-D-arabofuranoside has R_G 0·73). The chloroform fraction yielded a non-reducing syrup (40 mg., 4%), which was not examined further.

Methyl 2 : 3-anhydro-β-D-lyxofuranoside (III; R = H) (8·4 g.) gave, after ammonolysis and appropriate treatment, methyl 3-deoxy-3-*iso*propylideneamino-β-D-arabofuranoside (IV; RR′ = CMe_2, X = H) (7·1 g., 62%). The residue (3·5 g.) was *N*-acetylated [9] and the syrupy product (3·4 g., 96%) condensed with dry acetone [2] as above, giving a non-reducing syrup (3·0 g.), $[\alpha]_D^{22}$ −48° (*c*, 1·5 in H_2O), which was fractionated between chloroform and water. The aqueous fraction gave a non-reducing syrup (2·65 g., 88·5%) which after distillation at 170—175°/0·1 mm. (0·94 g.) showed on chromatographic analysis spots with R_G 0·60 and 0·70 (methyl 3-acetamido-3-deoxy-β-D-arabofuranoside has R_G 0·65). A solution of this syrup in ethanol–light petroleum deposited crystals (60 mg.), m. p. 222—224°, $[\alpha]_D^{26}$ −64° (*c*, 0·9 in H_2O), R_G 0·60 (Found: C, 46·4; H, 7·4. $C_8H_{15}O_5N$ requires C, 46·8; H, 7·4%). A solution of the crystals (13·5 mg.) in hydrochloric acid (10 ml.; 3N) was heated at 100° for 30 min. Concentration to dryness gave a syrup (12·2 mg.) which crystallised; the solid had $[\alpha]_D^{18}$ +40° (*c*, 0·60 in H_2O) (Wolfrom and Anno [4] record $[\alpha]_D$ +40° for 2-amino-2-deoxy-D-xylose hydrochloride).

The chloroform fraction yielded a dark brown syrup (70 mg., 2·3%) which was not examined further.

Methyl 3-*Acetamido*-3-*deoxy*-2 : 5-*di*-O-*methyl*-α- *and* -β-D-*arabofuranosides*.—Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside [5] (IV; R = H, R′ = Ac, X = H) (4·0 g.) was methylated four times with methyl iodide (23 ml.) and silver oxide (20 g.). The product, a mobile syrup (4·30 g.), after distillation at 50—120°/0·05 mm., had n_D^{20} 1·4647. This partly crystallised; the hygroscopic crystals (2·69 g., 59%) of *methyl* 3-*acetamido*-3-*deoxy*-2 : 5-*di*-O-*methyl*-α-D-*arabo*-

furanoside (VI) could not be recrystallised. Freed from syrup by cold light petroleum they had m. p. 57—59°, $[\alpha]_D^{23}$ +131° (*c*, 1·0 in H_2O), R_G 0·97 (Found : C, 51·6; H, 8·1; N, 5·8; OMe, 39·2. $C_{10}H_{19}O_5N$ requires C, 51·5; H, 8·2; N, 6·0; OMe, 39·9%).

Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside[5] (IV; R = H, R′ = Ac, X = H) (2·0 g.) in methanol (15 ml.) after three methylations with Purdie's reagents gave a product (0·90 g.), which was dissolved in acetone. Cooling the solution gave crystals *A* (580 mg.). Addition of light petroleum caused the deposition crystals *B* (280 mg.). Crystals *A* were non-reducing and after recrystallisation (acetone) depressed the m. p. of the starting material, crystals *B*, and methyl 3-acetamido-3-deoxy-5-*O*-methyl-β-D-arabofuranoside. The crystals, m. p. 157°, were therefore *methyl 3-acetamido-3-deoxy*-2-O-*methyl*-β-D-*arabofuranoside*, $[\alpha]_D^{24}$ −118° (*c*, 0·7 in H_2O), R_G 0·77 (Found : C, 49·7; H, 7·9; N, 6·3; OMe, 28·3. $C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4; OMe, 28·3%). Tosylation[8] (50 mg.) gave a crystalline toluene-*p*-sulphonate (55 mg.), m. p. 85—86°, $[\alpha]_D^{22}$ −69° (*c*, 0·9 in $CHCl_3$), which gave a positive test for a primary toluene-*p*-sulphonyloxy-group.[6]

Crystals *B* were also non-reducing, and after recrystallisation (acetone–light petroleum) had m. p. 156—160° (Found : C, 51·0; H, 8·5; N, 5·0; OMe, 36·2. $C_{10}H_{19}O_5N$ requires C, 51·5; H, 8·2; N, 6·0; OMe, 39·9%). The m. p.s of starting material, crystals *A*, and methyl 3-acetamido-3-deoxy-5-*O*-methyl-β-D-arabofuranoside were all depressed. Chromatographic analysis showed two spots, R_G 0·92 and R_G 0·78.

The residue (1·1 g.; OMe, 24·9%) left after the separation of crystals *A* and *B* was thrice methylated with Purdie's reagents. The product (1·0 g.; OMe, 36·5%) was extracted with cold acetone (2 ml.). Addition of light petroleum gave needles of *methyl 3-acetamido-3-deoxy*-2 : 5-*di*-O-*methyl*-β-D-*arabofuranoside* (VI) (0·51 g.) which after recrystallisation had m. p. 174—175°, $[\alpha]_D^{23}$ −107° (*c*, 0·8 in H_2O), R_G 0·92 (Found : C, 51·6; H, 8·1; N, 5·9; OMe, 39·4. $C_{10}H_{19}O_5N$ requires C, 51·5; H, 8·2; N, 6·0; OMe, 39·9%).

Methyl 2 : 3-Anhydro-5-methyl-β-D-lyxofuranoside.—Methyl 2 : 3-anhydro-β-D-lyxofuranoside[2] (III; R = H) (8·0 g.), initially dissolved in dry methanol (15 ml.), was thrice methylated with Purdie's reagents. The product (7·3 g.), a very mobile syrup, $[\alpha]_D^{26}$ −84° (*c*, 0·7 in H_2O), gave on distillation (b. p. 54—57°/0·05 mm.) a syrup (6·4 g., 73%) which crystallised when cooled to 0° but melted at *ca.* 14—15°. It had $[\alpha]_D^{20}$ −88° (*c*, 1·1 in H_2O), n_D^{21} 1·4470 (Found : C, 52·8; H, 7·8; OMe, 39·1. $C_7H_{12}O_4$ requires C, 52·5; H, 7·6; OMe, 38·8%).

Ammonolysis of Methyl 2 : 3-Anhydro-5-O-methyl-α-D-lyxofuranoside.—A solution of methyl 2 : 3-anhydro-5-*O*-methyl-α-D-lyxofuranoside[1] (III; R = Me) (9·1 g.) in dry methanolic ammonia (500 ml.) was heated in an autoclave at 120° for 48 hr. The resulting brown syrup after filtration and treatment with charcoal was concentrated, and the product freed from ammonia by distillation with ethanol. The residue, a brown syrup (9·51 g., 94%), was dissolved in hot acetone and the solution cooled. Crystals (8·34 g., 72%), shown to be *methyl 3-deoxy-5-O-methyl*-3-iso*propylideneamino*-α-D-*arabofuranoside* (IV; RR′ = CMe_2, X = Me), were deposited and after several recrystallisations (acetone) had m. p. 115—125°, $[\alpha]_D^{22}$ +106° (*c*, 0·9 in H_2O) (Found : C, 54·3; H, 9·4; N, 6·4. $C_{10}H_{19}O_4N$ requires C, 55·3; H, 8·8; N, 6·5%). Concentration of the mother liquors gave a dark brown syrup (2·82 g.), $[\alpha]_D$ +65° (*c*, 0·7 in H_2O). Distillation at 100° (bath temp.)/0·03 mm. gave a syrup (520 mg.) from which more (80 mg.) of the crystals were isolated.

The crystalline *N-iso*propylidene derivative (5·81 g.) in water (150 ml.) was acidified with 2N-hydrochloric acid and *N*-acetylated.[9] The syrupy product (5·80 g., 99%) crystallised after distillation at 140°/0·05 mm. All attempts at recrystallisation failed; the hygroscopic *methyl 3-acetamido-3-deoxy*-5-O-*methyl*-α-D-*arabofuranoside* (3·87 g.) (IV; R = H, R′ = Ac, X = Me), freed from syrup by ether, had m. p. 60—62°, $[\alpha]_D^{21}$ +119° (*c*, 0·9 in H_2O), R_G 0·88 (Found : C, 48·2; H, 7·6; N, 5·9; OMe, 27·8. $C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4; OMe, 28·3%).

Four methylations with Purdie's reagents gave a mobile syrup (1·07 g. from 1·0 g.), n_D^{22} 1·4548. Distillation gave two fractions : (*a*) 310 mg., b. p. (bath temp.) 80°/0·02 mm., which was not examined further; and (*b*) 520 mg., b. p. 100°/0·02 mm., which crystallised. Recrystallisation was difficult but the crystals were freed from syrup with light petroleum. They had m. p. and mixed m. p. 57—59° with authentic methyl 3-acetamido-3-deoxy-2 : 5-di-*O*-methyl-α-D-arabofuranoside (VI), $[\alpha]_D$ +129° (*c*, 0·9 in H_2O), R_G 0·97 (Found : OMe, 39·1. Calc. for $C_{10}H_{19}O_5N$: OMe, 39·9%).

Ammonolysis of Methyl 2 : 3-Anhydro-5-O-methyl-β-D-lyxofuranoside.—A brown syrup (6·17 g., 90%) was obtained when methyl 2 : 3-anhdyro-5-*O*-methyl-β-D-lyxofuranoside (III; R = Me) (6·2 g.) was treated with methanolic ammonia (500 ml.) under the conditions used for the α-anomer. Attempts to prepare an *N-iso*propylidene[2] derivative gave, after distillation at

84—86°/0·03 mm., a syrup (5·64 g., 74%). *N*-Acetylation [8] gave a syrup (5·44 g., 96%) which on dissolution in acetone and addition of light petroleum deposited crystals (2·66 g., 48%), shown to be *methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside* (IV; R_2 = H, R′ = Ac, X = Me), m. p. 161—162° after recrystallisation (acetone–light petroleum), $[\alpha]_D^{23}$ −114° (*c*, 1·2 in H_2O), R_G 0·81 (Found: C, 49·5; H, 7·8; N, 5·8; OMe, 28·1. $C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4; OMe, 28·3%).

Evaporation of the mother liquors and distillation gave a syrup (71 mg.), $[\alpha]_D^{20}$ +30° (*c*, 0·7 in H_2O). An aqueous extract of the distillation residues yielded another syrup (80 mg.), $[\alpha]_D^{23}$ +18° (*c*, 0·8 in H_2O).

Methyl 3-acetamido-3-deoxy-5-*O*-methyl-β-D-arabofuranoside (IV; R = H, R′ = Ac, X = Me) (1·0 g.) in methanol (5 ml.) was methylated four times with Purdie's reagents. The product (1·0 g.) was dissolved in acetone and light petroleum added, giving methyl 3-acetamido-3-deoxy-2 : 5-di-*O*-methyl-β-D-arabofuranoside (VI) identical with that synthesised as above. The samples had m. p. and mixed m. p. with authentic material 174—175°, $[\alpha]_D^{23}$ −107° (*c*, 1·1 in H_2O), R_G 0·92 (Found: OMe, 40·1. Calc. for $C_{10}H_{19}O_5N$: OMe, 39·9%.

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419. *Methylene Derivatives of* L-*Fucose.*

By R. L. Nelson and Elizabeth Percival.

L-Fucose on treatment with paraformaldehyde yielded five methylene derivatives. Three of these have been separated and characterised as a di-*O*-methylene-L-fucose, and two mono-*O*-methylene-L-fucoses. 2-*O*-Methyl-L-fucose has been synthesised from methyl 3 : 4-*O*-*iso*propylidene-α-L-fucoside.

L-Fucose when treated with paraformaldehyde according to the method of Andrews, Hough, and Jones [1] gave chromatographic evidence that at least four compounds were formed. Crystals (I) were obtained from a rapidly-obtained chloroform extract of the neutralised product. The residual material, after further treatment with paraldehyde, was separated on cellulose into four fractions, and two of these fractions (III and IV) were investigated.

The crystals (I) (39·7% yield) which could not be detected on the paper chromatogram were non-reducing to Fehling solution and on hydrolysis gave L-fucose and 1·9 mol. of formaldehyde. They gave correct analytical results for a di-*O*-methylenefucose and on partial acid hydrolysis gave a mixture of L-fucose and fraction (IV). Separation on thick paper gave crystalline fraction (IV).

Fraction (IV) a mono-*O*-methylenefucose, was reducing and gave one mol. of formaldehyde on hydrolysis. Oxidation with periodate gave an uptake of 0·93 mol. of periodate and a release of 0·88 mol. of formic acid. Methylation and hydrolysis followed by separation on thick paper led to isolation of 2-*O*-methyl-L-fucose. Fraction (IV) must therefore be either the 3 : 5- or the 3 : 4-mono-*O*-methylene-L-fucose. Its high negative rotation ($[\alpha]_D^{20}$ −95°) is suggestive of a pyranose ring {cf. 2 : 3 : 4-tri-*O*-methyl-L-fucose, $[\alpha]_D$ −128°; [2] 2 : 3 : 5-tri-*O*-methyl-L-fucose, $[\alpha]_D^{20}$ +70° (*c*, 1·7 in H_2O) (unpublished work)}. If fraction (IV) is the 3 : 4-mono-*O*-methylene-L-fucose and not the 3 : 5-isomer, then fraction (I) is 1 : 2-3 : 4-di-*O*-methylene-L-fucose.

Fraction (III) was a reducing syrup; it gave approximately 1 mol. of formaldehyde on hydrolysis and was unaffected by periodate. Methylation followed by hydrolysis yielded a syrup which was shown to contain fucose, 3-*O*-methylfucose, and a substance with an R_F value slightly lower than that of 2 : 3 : 4-tri-*O*-methylfucose (paper chromatography). It would appear that the methylene group had been partly removed during methylation, and fraction (III) is tentatively identified as 2 : 5-mono-*O*-methylene-L-fucose.

Although fractions (II) and (V) were insufficient for complete investigation they both appeared to be mono-*O*-methylene derivatives. In contrast to rhamnose [1] no evidence for the presence of *O*-dimethyleneoxy-compounds was obtained.

Authentic crystalline 2-*O*-methyl-L-fucose has been synthesised from methyl 3 : 4-*O*-*iso*propylidene-α-L-fucoside by methylation followed by hydrolysis.

Experimental

Chromatography was carried out by the descending method on Whatman No. 1 filter paper, butane-1-ol–ethanol–water (40 : 11 : 19 v/v; upper layer) being used as mobile phase. L-Fucose and its derivatives were located on the chromatogram by spraying it with aniline oxalate. The rate of movement of compounds on the chromatogram is quoted relative to that of the solvent front (R_F value) and to that of L-fucose (R_{Fu} value).

Preparation of the Methylene Derivatives.—L-Fucose ($[\alpha]_D$ −74·5° in H_2O) (15 g.) was treated with paraformaldehyde according to the method of Andrews, Hough, and Jones.[1] After neutralisation with barium carbonate, the filtrate was rapidly extracted with chloroform (3 × 50 c.c.). The chloroform extracts were dried ($MgSO_4$) and evaporated to a syrup which

was dissolved in water and rapidly extracted with chloroform. The chloroform extracts on concentration gave crystals (3·3 g.) of material which could not be detected on the paper chromatogram. Recrystallisation from ethanol and sublimation afforded *di*-O-*methylene*-L-*fucose* (I), m. p. 77°, $[\alpha]_D$ +58° (*c*, 0·7 in MeOH), +68° (*c*, 0·87 in H_2O) (Found : C, 51·3; H, 6·3. $C_8H_{12}O_5$ requires C, 51·1; H, 6·4%). The combined aqueous residues were extracted with chloroform during 18 hr. with continuous shaking. Evaporation of the dried ($MgSO_4$) chloroform extracts gave a syrup (*A*). This syrup smelled strongly of formaldehyde and trioxan sublimed from it when it was heated under reduced pressure. Chromatographic examination showed the presence of compounds with R_F 0·80, R_{Fu} 3·5 (II); R_F 0·70, R_{Fu} 3·0 (III); R_F 0·60, R_{Fu} 2·4 (IV). Continuous extraction of the aqueous mother liquors with chloroform for a further 24 hr. yielded a syrup (*B*) which on chromatographic examination was shown to contain fractions (III) and (IV) with a trace of a compound (V), R_F 0·50. The aqueous mother liquors on evaporation gave a yellow syrup consisting mainly of fucose (chromatography). This syrup together with fucose (2 g.) was treated with paraformaldehyde as before.[1] More of fraction (I) (3·6 g.) (total yield 6·9 g. from 17 g. of fucose) was obtained. The aqueous solution on evaporation gave a yellow syrup (*C*).

Syrups *A*, *B*, and *C* were combined and a portion (*ca.* 5 g.) was fractionated on a cellulose column with light petroleum (b. p. 100—120°)–butane-1-ol (70 : 30, changed in stages to 40 : 60 parts v/v) as the mobile phase; finally the fucose was eluted with ethanol. The following fractions were collected and treated with charcoal : (II) Crystalline (37 mg.), $[\alpha]_D^{20}$ +12° (*c*, 0·37 in H_2O), non-reducing to Fehling solution. (IIa) Syrup (26 mg.), a mixture of (II) and (III). (III) Syrup (248 mg.), $[\alpha]_D^{25}$ +16°, reducing. (IV) Crystalline (677 mg.), m. p. 96°, reducing to Fehling solution, $[\alpha]_D$ −124° (2 min.) ⟶ −90·5° (const., *c*, 5·8 in H_2O) (Found : C, 47·6; H, 6·7. $C_7H_{12}O_5$ requires C, 47·7; H, 6·8%). (V) (25 mg.), $[\alpha]_D$ −64° (*c*, 0·25 in H_2O).

Hydrolysis of the Methylene Derivatives.—The crystalline derivatives and the syrups were hydrolysed with N-sulphuric acid at 100° for 2 hr. [2N-H_2SO_4; 4 hr. for fraction (I)] giving fucose (detected chromatographically) and formaldehyde. In each case, formaldehyde was identified and estimated according to the method used by Andrews, Hough, and Jones.[1] Results are given in Table 1.

TABLE 1.

Compound or fraction	I	II	IIa	III	IV	V
M (calc.)	188	176	176	176	176	176
Wt. taken (mg.)	10·89	9·2	13	9·5	10·9	12·3
Yield of dimedone deriv. (mg.)	31·6	10·0	20·4	13·4	17·8	17·6
Yield of formaldehyde (mol.) ...	1·90	0·66	0·95	0·86	1·0	0·86

Periodate Oxidation.—To each compound (5—10 mg.) in water (5 c.c.) 0·2N-sodium metaperiodate (2 c.c.) was added, and the mixture was set aside in the dark. After 3 hr. the reaction mixture was treated according to one of the following methods : (i) Boric acid (2 g.) and saturated borax (5 ml.) were added and the mixture was set aside (3 min.). Potassium iodide (5 ml., 40%) was then added and the solution titrated with sodium arsenite. (ii) Ethylene glycol (1 c.c.) was added, and the formic acid liberated was titrated with 0·01N-sodium hydroxide. In order to hydrolyse the formyl esters which had been produced the formic acid titrations were completed on hot solutions. Results are given in Table 2.

TABLE 2.

Compound or fraction	II	III	IV	Compound or fraction	II	III	IV
M (calc.)	176	176	176	Wt. taken (mg.)	9·0	9·5	7·70
Wt. taken (mg.)	9·0	9·5	6·8	Formic acid yield (c.c. of			
Periodate uptake (c.c. of 0·01N)	2·73	2·2	7·10	0·01N)	nil	0·5	3·0
,, (mol.)	0·25	0·19	0·93	,, ,, (mol.)	nil	0·13	0·88

Partial Hydrolysis of Di-O-methylene-L-fucose (I).—The compound (333 mg.) was heated at 100° in 0·01N-sulphuric acid and the hydrolysis followed polarimetrically: $[\alpha]_D$ +225° (initial), +219° (15 min.), +210° (2 hr.). The concentration of acid was increased to 0·02N and heating continued. The rotations observed were +180° (5 hr.), +141° (10 hr.), +110° (13 hr.). Aliquot portions were removed after (*a*) 5 hr., (*b*) 10 hr., and (*c*) 13 hr. Neutralisation, concentration, and chromatographic examination showed (*a*) a single faint spot, R_F 0·60, R_{Fu} 0·24 (cf. fraction IV), (*b*) a spot R_F 0·60, and a faint spot corresponding to fucose, (*c*) the same two

spots as (*b*). The remaining solution afforded a syrup after cooling, neutralisation ($BaCO_3$), and concentration. Separation of this syrup on thick paper gave two fractions: (1) R_F 0·60, R_{Fu} 0·24, crystalline, m. p. and mixed m. p. with compound (IV) 96°, $[\alpha]_D^{18}$ −95° (*c*, 0·25 in H_2O); (2) crystalline fucose, m. p. 140—142°.

Fraction (III).—This syrup (100 mg.) was methylated thrice with Purdie reagents. Hydrolysis with N-sulphuric acid for 2 hr., neutralisation ($BaCO_3$), and concentration gave a syrup (48 mg.) which gave spots corresponding to fucose (trace), 3-*O*-methylfucose, and a dark red spot with R_F 0·66 [cf. 2 : 3 : 4-tri-*O*-methylfucose, R_f 0·70; 2 : 3 : 5-tri-*O*-methylfucose, R_F 0·87 (unpublished work)] similar in colour to other spots given by fucofuranose derivatives.

3 : 4-O-*Methylene*-L-*fucose* (IV).—This compound (280 mg.) was methylated thrice with Purdie reagents. The addition of methanol (2 c.c.) was necessary to dissolve the material in the first methylation. The product, a syrup (n_D^{18} 1·4650), was hydrolysed for 1 hr. with N-sulphuric acid at 100° and the neutralised solution ($BaCO_3$) filtered and concentrated to syrup. Chromatographic examination showed this to be 2-*O*-methylfucose contaminated with a trace of fucose. Separation on thick paper gave crystalline 2-*O*-methyl-L-fucose, m. p. and mixed m. p. 150°.

Synthesis of 2-O-*Methylfucose* (With Miss B. Guarco).—Methyl 3 : 4-*O*-*iso*propylidene-α-L-fucoside was prepared by Percival and Percival's method [4] from L-fucose. Four methylations with Purdie reagents followed by distillation (bath temp. 90—100°/0·04 mm.) gave a syrup, n_D^{16} 1·4555°. Hydrolysis with 4% sulphuric acid at 100° for 6 hr. afforded crystalline 2-O-*methylfucose*, m. p. 150—152°, $[\alpha]_D^{18}$ −87° (*c*, 1·0 in H_2O) (Found: C, 48·0; H, 7·6; OMe, 17·55. $C_7H_{14}O_5$ requires C, 47·2; H, 7·8; OMe, 17·4%).

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345. *The Water-soluble Polysaccharides of* Cladophora rupestris. *Part II.*[1] *Barry Degradation and Methylation of the Degraded Polysaccharide.*

By J. J. O'Donnell and Elizabeth Percival.

Repeated successive treatment of the water-soluble polysaccharide, cladophoran, with periodate and with phenylhydrazine gave a 25% yield of a degraded polymer containing L-arabinose, D-galactose, and L-rhamnose and 15% of sulphate groups. Evidence is presented that sulphate groups are linked to sugar residues other than galactose. Methylation studies established the linkages present in the degraded material. The significance of these results in relation to the whole polymer is discussed.

The water-soluble extract from the green seaweed, *Cladophora rupestris*, is a heteropolysaccharide material, which failed to separate into simple polymers when subjected to the usual fractionation techniques. The presence of 20% of organically bound sulphate made complete methylation difficult. However, separation and characterisation of a number of the sugars in the hydrolysate of the methylated material revealed some of the linkages present in the original polysaccharide, although it was not possible to advance any general structure for the polysaccharide or to allocate the sulphate residues to any particular sugar.[1]

Repeated degradation by Barry's method [2] appeared to offer a means of obtaining information on the structure of the inner part of this molecule. A single Barry degradation involves oxidation of the polysaccharide with periodate, followed by treatment of the oxo-polysaccharide with phenylhydrazine. This treatment removes all the residues in the original molecule which contained α-glycol groups and new vicinal hydroxyl groups are exposed in the degraded polymer. The process can then be repeated, and in this way successive layers of residues are removed from the periphery of the molecule. In the present experiments the reduction of periodate and the production of formic acid were measured for each oxidation. At the same time the oxo- and degraded polymers were isolated at each stage and their constituent sugars and sulphate and nitrogen contents were determined (see Tables 1 and 2).

Table 1.

	IO_4 reduced (moles/kg.)	Formic acid (moles/kg.)	Yield g.	Yield % †	N (%)
O_1 *	2·82	1·8	18·2	89	—
O_1D_1	—	—	13·7	63	3·5
D_1O_2	1·97	0·9	9·9	80	2·7
O_2D_2	—	—	6·3	66	4·2
D_2O_3	2·52	1·0	4·8	73	2·4

* O and D refer to the respective oxo- and degraded polysaccharides isolated. The second letter indicates the nature of the last treatment and the subscripts correspond to the number of such treatments.

† In calculating percentage yields of oxo-polysaccharide, allowance is made for material consumed during measurements.

Analysis of the hydrolysate of the oxo-polysaccharide O_1 confirmed the cleavage of all the xylose and a considerable proportion of the galactose units. For these sugars to be attacked by periodate they must be present as end-group or linked only through positions 1 and 4 (and/or 6 in the case of galactose) in the original polysaccharide. Glyoxal bisphenylhydrazone was isolated from the ethereal extract of the degraded material O_1D_1. This is in keeping with the cleavage of 1 : 4-linked xylose and galactose residues. No

TABLE 2.

	Molar proportions of sugars [3]					Ash (%)	SO_4 (%)	N (%)
	Arabinose	Galactose	Glucose	Xylose	Rhamnose			
Cladophoran	4·0	3·0	0·23	1·1	0·5	15·7	18·3	1·7
O_1	4·0	0·88	0·35	—	0·67	14·9	19·2	1·5
O_1D_1 *	—	—	—	—	—	15·6	16·6	3·5
D_1O_2	4·0	0·89	—	—	0·65	12·3	17·5	2·7
O_2D_2 *	—	—	—	—	—	12·8	16·9	4·2
D_2O_3	1·0	1·04	—	—	0·56	13·4	15·2	2·4

* A sample hydrolysate of the degraded material was chromatographically indistinguishable from that of the oxo-polysaccharide (visual examination).

other phenylhydrazone fragments were detected in this extract, although the presence of a large amount of *N*-acetylphenylhydrazine may be responsible for the failure to detect glycerosazone which has very similar solubility and chromatographic properties.

The second oxidation consumed one mole of periodate for every three sugar residues. Apparently all the glucose residues (*ca.* 5% of the molecule) were attacked, since this sugar was absent from the hydrolysate of the oxo-polysaccharide D_1O_2. The relative proportions of the other sugars were unchanged, and they must therefore have been attacked to approximately the same extent in this second oxidation. The disappearance of glucose, which was verified by a second experiment, is difficult to understand. Earlier work on cladophoran had indicated that at least part of the glucose was present as a separate 1 : 3-linked glucan. This would be immune to periodate attack, except at the ends of the chains. It may be that the glucan is a small, highly branched molecule with other linkages also present and that two oxidations are necessary to remove the branches and leave a linear molecule of dialysable proportions.

During the third oxidation, a large proportion of the arabinose residues were attacked, and an oxo-polysaccharide D_2O_3 containing arabinose : galactose : rhamnose in the molar proportions of 1 : 1 : 0·56 and representing *ca.* 25% of the original material was isolated.

Degradation removes those units from the polymer that have previously been attacked by periodate. The calculated yields of O_1D_1 and of O_2D_2, based on this loss, are in good agreement with the actual yields of the respective degraded polymers (see Table 1). Although the nature of the nitrogen-containing groups in the degraded material is uncertain, they are apparently attacked by periodate since the nitrogen content is reduced on oxidation.

Complete methylation of the degraded polymer D_2O_3 was carried out by the method of Fear and Menzies.[4] After hydrolysis of the methylated material with hydrochloric acid, and neutralisation with silver carbonate, the mixture of neutral methylated sugars and inorganic salts was evaporated to dryness. Exhaustive extraction of the residue with water was efficacious in leaving brown nitrogenous material adsorbed on the precipitated silver salts. The neutral methylated sugars were separated on a cellulose column, and the remainder of the contaminating nitrogenous material was removed in the first fraction as a fast-flowing mixture which was discarded. 2 : 4-Di-*O*-methylrhamnose (1 part), 2 : 4-di-*O*-methylarabinose (4 parts), 2-*O*-methylarabinose (1 part), D-galactose (1 part), 2 : 4 : 6-tri-*O*-methylgalactose (3 parts), 6-*O*-methylgalactose (1 part) and L-rhamnose (3 parts) were separated and characterised. None of these sugars has methoxyl groups on adjacent carbon atoms. This is in keeping with the immunity of the material D_2O_3 to further attack by periodate. With the exception of the proportion of rhamnose, which is somewhat higher in the hydrolysate of the methylated material, the relative proportions of the methylated derivatives are in good agreement with those of the sugars in the degraded material D_2O_3 (Table 2).

Earlier studies [1] revealed the presence of galactofuranose residues in cladophoran. While it is recognised that the present experiments have not eliminated the possibility of the presence of arabo- and rhamno-furanose residues, the resistance of these units in the polysaccharide to mild acid-hydrolysis is in favour of a pyranose structure. Therefore,

all the available evidence being borne in mind, the linkages which may be present in the centre of the cladophoran molecule are:

..3Rl..	...3Al..	..3Al.. 4	..3Gal.. 4 6	..3Rl.. 4	3Gal.. 4	..3Gal..
(1)	(4)	(1)	(1)	(3)	(1)	(3)

R = rhamnose, A = arabinose, Ga = galactose.

Comparison of these methylation results with those from the whole polymer show that the first four sugars given above are common to the hydrolysates of both methylated materials. Of the remaining derivatives isolated from the methylated degraded hydrolysate all except the L-rhamnose could have been formed, from lesser methylated derivatives present in the undegraded hydrolysate, by methylation of hydroxyl groups set free during the degradation. The failure to separate any free rhamnose from the hydrolysate of the methylated whole cladophoran is not very surprising when it is remembered that the total rhamnose content corresponded to *ca.* 5%, and that some seventeen different sugar derivatives were shown to be present in this hydrolysate including three different methylated rhamnoses.

The sulphate content of the various degraded products remained approximately constant, indicating that the sulphate ester groupings are spread relatively evenly throughout the molecule. The sulphate content of the degraded cladophoran D_2O_3 is equivalent to about one sulphate group per 3 or 4 sugar residues, and earlier work [1] has shown that this is not reduced on methylation. The 1 : 3-linked galactose is unlikely to be present as end group carrying sulphate on $C_{(3)}$, as such sulphate would be labile in the alkaline conditions of methylation and give rise to 3 : 6-anhydrogalactose.[5] If the sulphate groups are linked to galactose at all then, since they are not labile to alkali, they are probably located at position 4. Only one residue in seven of the sugars present in the hydrolysate of the methylated degraded material is a galactose unit with $O_{(4)}$ available for linkage. At least some of the sulphate groups must therefore be carried by either arabinose and/or rhamnose. This is the first evidence of sulphate groups linked to residues other than galactose in seaweed polysaccharides. The large amount of quadruply linked rhamnose in the polymer makes this sugar a very likely site for these residues, but a definite answer to this question must await other methods of investigation.

Certain broad conclusions can be drawn from these experiments: the material has a highly branched structure, with xylose and galactose units at the ends of the branches, whereas galactose, arabinose, and rhamnose residues comprise the centre of the molecule. Sulphate groups are linked to residues both on the outer branches and in the centre of the molecule. Finally, after three oxidations and two treatments with phenylhydrazine the molecule is still sufficiently large to be retained by a dialysis sac.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper. Paper ionophoresis [6] was done in borate buffer (pH 10) at 750 v for 5 hr. Evaporations were carried out at 40°/15 mm. Specific rotations were measured in water at 18°.

The water-soluble polysaccharide, cladophoran (26·3 g.), $[\alpha]_D$ +69° (ash, 13·7%), was alternately oxidised with periodate and degraded with phenylhydrazine three times. In a typical experiment the polysaccharide (4% concentration) was treated in the dark at room temperature with 0·1M-sodium metaperiodate and the course of the reaction followed by measuring the periodate reduced [7] and the formic acid released [8] on samples (1 c.c.) withdrawn at intervals (see Table 1). After 50 hr. the reaction had reached completion, and the excess of periodate was removed with sulphur dioxide and the mixture dialysed until free from inorganic ions. An aliquot part was removed and hydrolysed with N-sulphuric acid at 100° for 6 hr. The molar proportions of the sugars in the resulting syrup were determined by Pridham's method [3] (see Table 2). For this method of estimation a synthetic mixture of the sugars present in the hydrolysate was made by dissolving millimolar quantities of these sugars in 25 c.c. of water.

Measured quantities of this mixture (0·02, 0·04, 0·06, 0·08 c.c.) were applied from an Agla microburette at intervals along the starting line of a paper chromatogram (20 × 40 cm.). Also spotted on the paper were samples of the hydrolysate. After elution the chromatogram was sprayed with a 1% solution of freshly prepared *p*-anisidine hydrochloride and heated at 130° for 10 min. Each of the coloured spots which developed, together with suitable blanks, were cut out and left for 10 min. in 3 c.c. of methanolic stannous chloride (1 g. of stannous chloride dissolved in 5 c.c. of water, 90 c.c. of methanol added, and the mixture filtered). The density of the resulting solution was then measured in a Unicam spectrophotometer at the wavelength of maximum absorbance for the particular sugar being examined. When the readings for the standard solutions of the respective sugars were plotted against concentration a straight-line graph was obtained in each case, and these were used to determine the unknown materials. A complete duplication of the experiment ensured accuracy within ±5%.

The oxo-polysaccharide in the remainder of the solution was isolated by freeze-drying. The second oxidation was not complete until after 70 hr. and the third oxidation after 86 hr.

The oxo-polysaccharide was degraded by heating a 4% aqueous solution at 100° for 2 hr. with 7% acetic acid and 3% phenylhydrazine. After exhaustive extraction with ether and dialysis the degraded polysaccharide was isolated, by freeze-drying, as a light yellow powder. Further extraction with ether failed to reduce the nitrogen content.

The ethereal extracts after evaporation to dryness and extraction with glacial acetic acid afforded a brown powder. Dissolution in ether and addition of light petroleum afforded yellow crystals of glyoxal bisphenylhydrazone (0·9 g.), m. p. and mixed m. p. 167°. Removal of the acetic acid yielded crystalline *N*-acetylphenylhydrazine, m. p. and mixed m. p. 124°.

Methylation of the Degraded Cladophoran D_2O_3.—Thallium hydroxide (6 g.) and an aqueous solution of cladophoran D_2O_3 (3·6 g.; 30 c.c.) were freeze-dried and the product was refluxed overnight with methyl iodide (25 c.c.).[4] After evaporation to dryness the residue was exhaustively extracted with methanol (3 × 25 c.c.), hot 50% aqueous methanol (3 × 25 c.c.), and hot water (3 × 25 c.c.). The combined extracts were re-treated with thallium hydroxide and methyl iodide. After four methylations in all, the final residue was exhaustively extracted with chloroform. Removal of the chloroform from the combined extracts gave a brown powder (1·88 g.) (Found: OMe, 26·7%). Several methylations with Purdie reagents failed to raise the methoxyl content above 26·9%.

Hydrolysis of the Methylated Polysaccharide and Characterisation of the Methylated Sugars.—The above powder (1·80 g.) was hydrolysed under reflux with N-methanolic hydrogen chloride–water (9 : 1 by vol.) for 7 hr. Following neutralisation with silver carbonate and evaporation to dryness, the residue was thoroughly extracted with water. Removal of the water from the aqueous extracts and chloroform-extraction of the residue gave on evaporation an amorphous hydrolysate (0·96 g.). This was separated into its constituents on a cellulose column (55 × 2·3 cm.). After elution of five fractions with a water-saturated mixture of light petroleum (b. p. 60—80°)–butan-1-ol (8 : 2), the proportions were changed to 7 : 3. R_G values are recorded for paper chromatograms developed with butan-1-ol–ethanol–water (4 : 1 : 5). The products of demethylation [1] were detected by paper chromatography. The following fractions were collected:

Fraction 1, a yellow syrup (213 mg.) of R_G 1·05 (Found: N, 14·3%).

Fraction 2, syrupy 2 : 4-di-*O*-methylrhamnose (40 mg.), R_G 0·83, $[\alpha]_D$ −17° (*c* 0·3) (Found: OMe, 33·6. Calc. for $C_8H_{16}O_5$: OMe, 32·3%); demethylation gave only rhamnose; nucleation with an authentic specimen afforded needles of 2 : 4-di-*O*-methylrhamnose [9] with m. p. and mixed m. p. 82—83°.

Fraction 3, syrupy 2 : 4-di-*O*-methylarabinose (162 mg.), R_G 0·67, $[\alpha]_D$ +28° (*c* 2·0) (Found: OMe, 34·9. Calc. for $C_7H_{14}O_5$: OMe, 34·8%); demethylation of a portion of the syrup gave only arabinose; the derived anilide [10] had m. p. 126° and gave an *X*-ray powder photograph identical with that of 2 : 4-di-*O*-methyl-*N*-phenyl-L-arabinosylamine.

Fraction 4, syrupy 2 : 4 : 6-tri-*O*-methylgalactose (143 mg.), R_G 0·64, which crystallised from methanol; the crystals had m. p. 116°, $[\alpha]_D$ +88° (*c* 0·92) [11] (Found: OMe, 41·7. Calc. for $C_9H_{18}O_6$: OMe, 41·9%); demethylation gave only galactose.

Fraction 5, syrupy 2-*O*-methylarabinose (37 mg.), R_G 0·43, $[\alpha]_D$ +74° (Found: OMe, 18·0. Calc. for $C_6H_{12}O_5$: OMe, 17·8%); this was chromatographically and ionophoretically identical with authentic 2-*O*-methylarabinose and in keeping with this structure failed to give a red spot on spraying of a paper chromatogram with triphenyltetrazolium hydroxide; [12] the derived phenylhydrazone had m. p. 115°.

Fraction 6, crystalline L-rhamnose (89 mg.), R_G 0·30, $[\alpha]_D$ +8·2 (const.), m. p. and mixed m. p. 68°.

Fraction 7, syrupy 6-*O*-methylgalactose (23 mg.), R_G 0·23, $[\alpha]_D$ +74° (*c* 1·1) (Found: OMe, 15·1. Calc. for $C_7H_{14}O_6$: OMe, 16·0%); demethylation gave only galactose; the derived phenylhydrazone had m. p. 117°.[13]

Fraction 8, crystalline D-galactose (46 mg.), R_G 0·10, $[\alpha]_D$ +80° (const.), m. p. and mixed m. p. 162—164°.

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283. *Methyl Ethers of* L-*Fucose.*

By J. G. Gardiner and Elizabeth Percival.

The four methyl L-fucosides have been synthesised and separated on cellulose. Partial methylation of methyl β-L-fucofuranoside and of methyl α-L-fucopyranoside followed by hydrolysis and separation on cellulose have led to the isolation of eight of the eleven possible methyl ethers of fucose.

The occurrence of L-fucose in a variety of natural polysaccharides led to this study of the properties of its methyl ethers. Treatment with 0·8% methanolic hydrogen chloride at room temperature until the rotation reached a maximum positive value gave the best yield (64·6%) of methyl α- and β-L-furanosides. The product after separation on a cellulose column gave methyl β-L-fucofuranoside (44%), α-L-fucofuranoside (20·6%), α-L-fucopyranoside (15·4%), and β-L-fucopyranoside (20%).

While this work was in progress Miss Watkins [1] recorded the separation of the four glycosides after treatment of fucose with hot 0·025% methanolic hydrogen chloride but she gave no indication of the relative proportions of the four products. We found that glycosidation in the hot gave a higher proportion of pyranosides.

Incomplete methylation of methyl β-L-fucofuranoside and of α-L-fucopyranoside with Purdie reagents followed by hydrolysis and partition on cellulose led to the isolation of the methylated fucoses shown in the Table.

	Fraction numbers †	From β-L-fucofuranoside (%)	Fraction numbers †	From α-L-fucopyranoside (%)
2 : 3 : 4-Tri-*O*-methyl-L-fucose	—	—	P1	5·5
2 : 3 : 5-Tri-*O*-methyl-L-fucose	F1	5·5	—	—
2 : 3-Di-*O*-methyl-L-fucose	F3	17·5	P2(a)	27·5
2 : 4-Di-*O*-methyl-L-fucose	—	—	P3	11·5
2 : 5-Di-*O*-methyl-L-fucose ..... } 3 : 5-Di-*O*-methyl-L-fucose * ..... }	F2	19·0	—	—
3 : 4-Di-*O*-methyl-L-fucose *	—	—	P4	2·5
5-*O*-Methyl-L-fucose *	F4	7·0	—	—
2-*O*-Methyl-L-fucose	F5	36·0	P5	43·5
3-*O*-Methyl-L-fucose *	F6	15·0	P7	9·5

† See Experimental.

Bell and Dedonder [2] have shown that sugars substituted at the position adjacent to the reducing group do not form a water-insoluble formazan with triphenyltetrazolium hydroxide and that a paper chromatogram sprayed with this reagent reveals only those reducing aldoses in which position 2 is unsubstituted. Only those derivatives marked with an asterisk in the Table gave red spots in this way.

The only methyl ether of L-fucose which was not obtained was the 4-*O*-methyl-L-fucose. In keeping with the recognised reactivity at position 2 in sugars it is noteworthy that the 2-*O*-mono- and 2 : 3-di-*O*-methyl ethers were obtained in highest yield from both methylations. Whereas the methyl ethers with a pyranose structure all had negative rotations, those containing a furanose ring were positive.

2 : 3 : 5-Tri-*O*-methyl-L-fucose was prepared by methylation of methyl β-L-fucofuranoside in dimethylformamide. Complete methylation proved difficult and after removal of the glycosidic methoxyl group the tri-*O*-methylfucose was separated from less methylated derivatives by partition on a cellulose column. It gave correct analyses for a tri-*O*-methyl-L-fucose and did not reduce periodate. It was an exceedingly hygroscopic and volatile syrup.

The first fraction (F1) separated as a syrup from the partition of the hydrolysate of the partly methylated furanoside, gave a single spot on ionophoresis, was chromato-

graphically identical with the above 2 : 3 : 5-tri-*O*-methyl-L-fucose, and did not reduce periodate. Owing to its high volatility it was exceedingly difficult to dry and a large proportion was lost in an attempt to dry it over phosphoric oxide in a vacuum-desiccator. Consequently the recorded rotation and methoxyl contents are on moisture-containing material.

Fraction F3 and fraction P2(a) (Table) had $[\alpha]_D$ −97° and −100° respectively (cf. 2 : 3 : 4-tri-*O*-methylfucose, $[\alpha]_D$ −128°). They both gave correct analyses for di-*O*-methyl derivatives and had identical properties. The only dimethylfucose which can be synthesised from both a fucofuranoside and a fucopyranoside is the 2 : 3-di-*O*-methyl derivative. Further proof of the structure was obtained by oxidative preparation of D(−)-dimethoxysuccinic acid and its crystalline amide. The syrupy lactone, $[\alpha]_D$ +10° ⟶ +50° (96 hr.), isolated after oxidation of the dimethyl-sugar with bromine water was undoubtedly a furanolactone.[3] It is clear that the supposed 2 : 3-di-*O*-methyl-L-fucose ($[\alpha]$ +4·6°) isolated from methylated fucoidin by Conchie and Percival [3] by hydrolysis of fraction B contained a considerable quantity of impurity. Indeed these authors comment on the very low yield of D(−)-dimethoxysuccindiamide they isolated after oxidation of their material.

From the fucopyranoside hydrolysate crystalline 3 : 4- and 2 : 4-di-*O*-methyl-L-fucose were also separated. The former was identical with authentic material.[4] This 2 : 4-di-*O*-methyl-L-fucose was characterised by its methoxyl content and its resistance to periodate. The rotation ($[\alpha]_D$ −27° ⟶ −15°) of the derived lactone indicated a 1 : 5-ring structure. The di-*O*-methylfucose isolated from the methylated extracellular polysaccharide of *Aerobacter aerogenes* by Aspinall, Jamieson, and Wilkinson [5] and thought from the available evidence to be the 3 : 5-di-*O*-methyl-L-fucose has now been shown to be 2 : 4-di-*O*-methyl-L-fucose.

Fraction F2 (Table) gave a single spot on a paper chromatogram irrigated with solvents (1), (2), or (3) (see p.), gave a red spot on a paper chromatogram sprayed with triphenyltetrazolium hydroxide, and gave analyses correct for a di-*O*-methylfucose. However, on paper ionophoresis [6] (borate buffer, pH 10) two spots were obtained with M_G 0·65 and 0·02. Foster [7] attributes high mobility under these conditions of ionophoresis to the presence of free hydroxyl groups on $C_{(1)}$ and $C_{(2)}$ and the ability of the substance to form complexes across these two atoms. He has shown that neither 2 : 3- nor 2 : 4-di-*O*-methylrhamnose ($M_G < 0{\cdot}05$) has an appreciable mobility and that of the 2 : 3-, 2 : 4-, and 3 : 4-di-*O*-methylglucoses only the latter has an M_G (0·31) at all comparable with that recorded by us for this fraction. It appears therefore that the spot of M_G 0·65 corresponds to the 3 : 5-di-*O*-methyl-L-fucose and that the spot of M_G 0·02 is due to the presence of some 2 : 5-di-*O*-methyl-L-fucose.

Crystalline 2-*O*-methyl- [8] and 3-*O*-methyl-L-fucose [3] (the latter crystalline for the first time) were isolated from the hydrolysates of both methylated fucosides. In addition 5-*O*-methyl-L-fucose has been separated and characterised.

The reduction of periodate by each of the methylated derivatives was measured.[9] 2 : 3 : 5-Tri-*O*-methyl- and 2 : 4-di-*O*-methyl-fucose, in keeping with their structures, were not oxidised; of the remaining derivatives only the mixture of 2 : 5- and 3 : 5-dimethyl ethers and the 3 : 4-di-*O*-methylfucose were reduced in an approximately theoretical manner. Anomalous results have previously been obtained for partly methylated 6-deoxyhexoses [3,4] and hexoses.[10]

Experimental

Evaporations were done at 40° under reduced pressure. Paper-partition chromatography was done on Whatman No. 1 filter paper with the upper layers of the following v/v solvent systems: (1) butan-1-ol–ethanol–water (4 : 1 : 5), (2) benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3), (3) ethyl methyl ketone half saturated with water plus ammonia (99 : 1); and the reducing sugars were located by aniline oxalate (AO), and the non-reducing sugars with aniline

oxalate containing 3% (v/v) syrupy phosphoric acid (AP). Methylated sugars in which position 2 was unsubstituted were revealed by triphenyltetrazolium hydroxide.[11] R_G, R_F, and R_{fu} are the rates of travel relative to tetramethylglucose, the solvent front, and fucose respectively. Ionophoresis was carried out according to the conditions used by Percival and Fisher.[6] Rotations were measured in water at 18°.

Preparation of Methyl L-*Fucosides.*—(A) L-Fucose (m. p. 145°) (10 g.) was dissolved in 0·8% methanolic hydrogen chloride (400 c.c.) at 15° and the change in rotation followed polarimetrically to a maximum $[\alpha]_D$ of +10·4° (66 hr.). Neutralisation with silver carbonate and evaporation of the filtrate gave a syrup (10·80 g.).

(B) L-Fucose (2 g.) in dry methanol (80 c.c.) was agitated with Amberlite resin (IR-100H) (3 g.) at 15°. After 13 days the solution had a maximum rotation of $[\alpha]_D$ +8·0°. Removal of solvent gave a syrup (2·19 g.). Paper chromatography of both syrups with solvent (1) for 40 hr. and spray (AP) showed 4 components with R_F 0·57, 0·51, 0·44, and 0·39 respectively. In addition, the syrup B, $[\alpha]_D$ +8·0°, contained a little free fucose.

The syrup A (2 g.) was separated on a cellulose column (85 × 2·7 cm.) by using ethyl methyl ketone saturated with water. Four fractions were collected and their R_G values measured with solvent (1). Fractions II, III, and IV were recrystallised from methanol: fraction I (0·7—1·1 l.), syrupy methyl β-L-fucofuranoside (0·86 g.), R_G 0·70, $[\alpha]_D$ +112° (*c* 7·0); fraction II (1·6—1·92 l.), methyl α-L-fucofuranoside (0·40 g.), R_G 0·62, m. p. 127—128°, $[\alpha]_D$ −108° (*c* 2·0), −115° (*c* 2·0 in MeOH); fraction III (2·14—2·44 l.), methyl α-L-fucopyranoside (0·30 g.), R_G 0·53, m. p. 158—159°, $[\alpha]_D$ −191° (*c* 2·0); fraction IV (2·551—4·0 l.), methyl β-L-fucopyranoside (0·38 g.), R_G 0·48, m. p. 126—127°, $[\alpha]_D$ +10·5 (*c* 1·0). After addition of ethyl methyl ketone (50 c.c.) the initial syrup A, $[\alpha]_D$ +10·5° (5 g.), deposited crystals of methyl α-L-fucopyranoside (0·47 g.), m. p. and mixed m. p. 158—159°.

Partial Methylation of Methyl β-L-*Fucofuranoside.*—The furanoside (3 g.) was methylated twice with methyl iodide (60 c.c.) and silver oxide (11·5 g.) at 45°. The product was heated at 100° with 0·3N-sulphuric acid (150 c.c.) until the rotation was constant (45 min.). Neutralisation, with barium carbonate, of the cooled solution, filtration, and evaporation gave a syrup (2·6 g.). Chromatographic analysis [solvent (1)] showed 7 spots. This syrup (2·5 g.) was separated on a cellulose column (85 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, v/v) saturated with water. After 3 l. had been collected the ratio of solvents was changed to 6·5 : 3·5, after another 2·5 l. to 6 : 4, and after another 1·5 l. finally to 1 : 1. The following fractions were collected. Each fraction appeared to be homogeneous and gave a single spot on paper chromatograms run in solvents (1), (2), and (3). The R_G values given are for solvent (1).

Fraction F1 (0·60—1·28 l.), a hygroscopic syrup (0·109 g.), R_G 1·05, $[\alpha]_D$ +47° (*c* 1·0), M_G 0·0 {2 : 3 : 5-tri-*O*-methyl-L-fucose had R_G 1·05, $[\alpha]_D$ +70° (*c* 1·3)} (Found: OMe, 36·3. Calc. for $C_9H_{18}O_5$: OMe, 45·1%); this syrup showed no appreciable reduction of periodate on prolonged standing with the oxidant.

Fraction F2 (1·30—2·00 l.), a syrup (0·384 g.), had R_G 0·92, M_G (two components) 0·65, 0·02, $[\alpha]_D$ +38° (*c* 2·5) (Found: OMe, 32·3. Calc. for $C_8H_{16}O_5$: OMe, 32·0%). One mole of this dimethyl sugar reduced the following number of moles of periodate: 0·36 (18 hr.); 0·46 (42 hr.); 0·71 (90 hr.); 1·04 (180 hr.). A portion of the syrup (90 mg.) was oxidised with bromine water at 37°. After 5 days the solution no longer reduced Fehling's solution. Treatment in the usual manner gave a mixture of syrupy lactones, $[\alpha]_D$ +20° ⟶ +36°.

Fraction F3 (2·50—3·50 l.), syrupy 2 : 3-*di*-O-*methyl*-L-*fucose* (0·3516 g.), had R_G 0·75, $[\alpha]_D$ −97° (*c* 3·0) (Found: OMe, 31·5. $C_8H_{16}O_5$ requires OMe, 32·05%). One mole reduced 0·23 (18 hr.), 0·39 (42 hr.), 0·64 (90 hr.), 0·82 (180 hr.) mole of periodate. Oxidation of a portion (0·05 g.) with bromine water at 37° for 6 days gave a syrupy lactone which had $[\alpha]_D$ +10° ⟶ +50° (96 hr.) (*c* 2·0). A portion (0·409 g.) was converted into D(−)-αβ-dimethoxysuccindiamide by oxidation first with 0·6M-sodium metaperiodate (25 c.c.) and then with bromine (7 days) according to the conditions used by Arni and Percival [12] for the oxidation of 3 : 4-di-*O*-methylfructose. Distillation of the ester (0·23 g.) (bath temp. 150°/0·05 mm.) gave a mobile syrup (0·158 g.), n_D^{20} 1·4318, $[\alpha]_D$ −73° (*c* 1·0 in MeOH). The derived amide (67 mg. from 97 mg.) had $[\alpha]_D$ −90° (*c* 0·6), m. p. and mixed m. p. with D(−)-dimethoxysuccindiamide 278°. Mixed m. p. with (±)-dimethoxysuccindiamide 240—246°.

Fraction F4 (3·92—4·56 l.), syrupy 5-O-*methyl*-L-*fucose* (0·1404 g.), had R_G 0·70, $[\alpha]_D$ +28·3° (*c* 0·8) (Found: OMe, 17·2. $C_7H_{14}O_5$ requires OMe, 17·4%). One mole reduced 1·83 (0·5 hr.),

1·99 (1·75 hr.), 2·22 (5·75 hr.), 2·70 (25 hr.), 2·70 (48 hr.) moles of periodate. The derived osazone had m. p. 190° and gave a positive test for OMe.

Fraction F5 (6·00—8·56 l.), 2-*O*-methylfucose (0·7262 g.), had R_G 0·60, m. p. and mixed m. p. 151°, $[\alpha]_D$ −87·2° (*c* 1·4). One mole reduced 1·04 (0·5 hr.), 1·17 (1·3 hr.), 1·30 (3·08 hr.), 1·98 (7 hr.), 2·93 (18 hr.), 3·8 (470 hr.) moles of periodate.

Fraction F6 (10·92—11·72 l.), syrupy 3-*O*-methyl-L-fucose (0·3056 g.), had R_G 0·48. This fraction crystallised when seeded with a crystal of Fraction P7 and had m. p. and mixed m. p. 110°, $[\alpha]_D$ −97° (*c* 1·0). One mole used 0·91 (18 hr.), 0·96 (42 hr.), 1·21 (90 hr.), 1·27 (160 hr.) moles of periodate. For further characterisation see Fraction P7.

Fucose (0·17 g.) was recovered from the water-washings of the column.

Partial Methylation of Methyl α-L-Fucopyranoside.—The pyranoside (5 g.) was methylated thrice with methyl iodide (40 c.c.) and silver oxide (10 g.) at 45°. Hydrolysis of the product as for the methylated furanoside gave a syrup (4·6 g.) which showed 7 spots on chromatography. The syrup was separated on a cellulose column with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, v/v) saturated with water. After 2·25 l. had been collected the ratio of solvents was changed to 6·5 : 3·5 and after another 1 l. had passed to 6 : 4 (5·75 l.). The R_G and R_F values of each fraction were measured in solvents (1) and (2) respectively.

Fraction P1 (1·00—1·40 l.), syrupy 2 : 3 : 4-tri-*O*-methyl-L-fucose (0·123 g.), had R_G 0·92, R_F 0·79, $[\alpha]_D$ −128° (*c* 1·1). Chromatography showed a single spot in solvents (1), (2), and (3), identical with those of authentic 2 : 3 : 4-tri-*O*-methyl-L-fucose. The derived glycoside after purification by sublimation had m. p. and mixed m. p. 95—96°, $[\alpha]_D$ −200° (*c* 1·0).

Fraction P2 (1·70—2·22 l.) was a syrup (0·928 g.), R_G 0·75, R_F 0·70, $[\alpha]_D$ −106° (*c* 7·7). Paper chromatography [solvent (3)] showed two spots, of R_F 0·47 and 0·32. This fraction (0·70 g.) was partitioned on a cellulose column (40 × 1·5 cm.) with solvent (3) and collected in 20 c.c. fractions. The solvent was removed from the respective fractions at 40° in a stream of nitrogen. Fraction P2(a) (180—240 c.c.) was syrupy 2 : 3-di-*O*-methyl-L-fucose (0·348 g.), $[\alpha]_D$ −95° ⟶ −100° (19 hr. const.) (*c*, 2·1). Chromatographic analysis showed a single spot in solvents (1), (2), and (3) identical with the spot given by fraction F3. The R_F in solvent (3) was 0·47 (Found: OMe, 30·5. Calc. for $C_8H_{16}O_5$: OMe, 32·2%). The syrup consumed 0·44 (43 hr.), 0·66 (55 hr.), 0·8 (160 hr.) mole of periodate per $C_8H_{16}O_5$ unit. Fraction P2(b) (260—280 c.c.) was a syrup (0·144 g.), shown by paper chromatography [solvent (3)] to be a mixture of fractions P2(a) and P2(c). Fraction P2(c) (300—400 c.c.) was a syrup (0·73 g.) which crystallised and had m. p. and mixed m. p. 131—132° with fraction P3 [recrystallised from chloroform–light petroleum (b. p. 40—60°)].

Fraction P3 (2·25—2·75 l.) was 2 : 4-*di*-O-*methyl-L-fucose* (0·127 g.), R_G 0·75, R_F 0·73, m. p. 131—132° [recrystallised from chloroform–light petroleum (b. p. 40—60°)], $[\alpha]_D$ −85° (*c* 0·85) (Found: OMe, 32·0%). This dimethyl-sugar was not oxidised by periodate during 300 hr.

Fraction P4 (3·15—4·00 l.) was 3 : 4-di-*O*-methyl-L-fucose (0·051 g.), R_G 0·7, R_F 0·67, m. p. and mixed m. p. 82° (cf. ref. 4). It moved at the same speed on a paper chromatogram as authentic 3 : 4-di-*O*-methyl-L-fucose in solvents (1), (2), and (3) and had $[\alpha]_D$ −118° (*c* 2·2). One mole took up 0·85 mole of periodate (55 hr.), 0·95 mole (160 hr.).

Fraction P5 (4·50—6·70 l.) was 2-*O*-methyl-L-fucose (0·932 g.), R_G 0·56, R_F 0·63, m. p. and mixed m. p. 150—152° (recrystallised from ethanol), $[\alpha]_D$ −68° (20 min.) ⟶ −85° (2·5 hr. const.) (*c* 1·0), −42° (*c* 1·0 in EtOH). The derived fucoside (2% MeOH–HCl under reflux) had $[\alpha]_D^{18}$ −57·7° (*c* 0·52) and reduced 0·45 (9 hr.), 0·67 (48 hr.) mole of periodate for every $C_8H_{16}O_5$ unit.

Fraction P6 (6·70—82·0 l.) was a colourless syrup (0·244 g.) shown by paper chromatography to be a mixture of fractions P5 and P7.

Fraction P7 (8·20—9·05 l.) was syrupy 3-*O*-methyl-L-fucose (0·59 g.), R_G 0·45, R_F 0·56, which crystallised slowly, then having m. p. 109—110°, $[\alpha]_D$ −97° (*c* 4·2), −60·8° (*c* 3·8 in EtOH) (Found: OMe, 17·8. Calc. for $C_7H_{14}O_5$: OMe, 17·4%). The derived osazone had m. p. 175°. The derived syrupy glycoside (2% MeOH–HCl under reflux) had $[\alpha]_D$ −66° (*c* 1·1) and did not reduce periodate.

Fraction P8 (9·05—10·50 l.) was fucose (0·6588 g.), m. p. and mixed m. p. 143°.

2 : 3 : 5-*Tri*-O-*methyl-L-fucose.*—Methyl β-L-fucofuranoside (0·60 g.), stirred vigorously with redistilled dimethylformamide (20 c.c.) and methyl iodide (5 c.c.), was treated with silver oxide (5 g.) added in small portions during 30 min.[13] The stirring was continued at room temperature for 17 hr. Inorganic salts were removed and washed with dimethylformamide (2 × 7 c.c.) and chloroform (4 × 15 c.c.), and residual silver salts were removed from the solution and washings

by agitation with 1% aqueous sodium cyanide (50 c.c.). The organic phase was separated and the aqueous phase extracted with chloroform (6 × 20 c.c.). The combined solution and extracts were concentrated to a syrup and the methylation was repeated (yield, 0·66 g. Found: OMe, 48·2%). Hydrolysis with 0·3N-sulphuric acid (50 c.c.) at 100° for 30 min. gave a reducing syrup (0·50 g.). Paper chromatography with solvent (1) showed three components with R_{fu} 2·31, 2·70, and 3·03 respectively. Separation of a portion (0·40 g.) on a cellulose column (38 × 2·2 cm.) with butan-1-ol–light-petroleum (b. p. 100—120°) (70 : 30 v/v) gave a fraction, syrupy 2 : 3 : 5-*tri*-O-*methyl*-L-*fucose* (0·27 g.) which gave a single pinkish-red spot on chromatography, R_{fu} 3·03 [solvent (1); spray AO], had n_D^{25} 1·4452, $[\alpha]_D^{18}$ +70° (*c* 1·5), +55° (*c* 1·4 in EtOH) (Found: OMe, 44·8. $C_9H_{18}O_5$ requires OMe, 45·1%), and did not reduce periodate.

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394. *The Structure of* Brachychiton diversifolium *Gum* (Sterculia caudata).

By E. L. Hirst, Elizabeth Percival, and R. S. Williams.

The gum exudate from *Brachychiton diversifolium* is an acetylated acidic polysaccharide which contains rhamnose (1 part), galactose (1 part), and glucuronic acid (2 parts). Graded hydrolysis of the gum gives D-glucurone, an aldobiuronic acid, identified as 2-*O*-α-D-glucuronosyl-L-rhamnose, and unidentified oligosaccharides of high uronic acid content.

The methylated gum gives on hydrolysis 2 : 3 : 4 : 6-tetra-*O*-methyl- and 2 : 3 : 6-tri-*O*-methyl-D-galactose, 3 : 4-di-*O*-methyl- and 3-*O*-methyl-L-rhamnose, 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, and a mixture of methylated uronic acids.

Through the kindness of Mr. V. Grenning, Director of Forests in Queensland, Australia, a sample of gum from *Brachychiton diversifolium* collected in the Atherton district of Northern Queensland was made available for study. This tree was formerly known as *Sterculia caudata*. Gum exudates of the *Sterculia* trees previously examined [1] have been found to be polymers of rhamnose, galactose, and galacturonic acid residues, partly acetylated in two instances at least. The gum from *Sterculia setigera* also contains residues of tagatose. The three species so far investigated, *S. urens*, *S. tormentosa*, and *S. setigera*, are, however, all characterised by a high uronic acid content and are consequently very resistant to hydrolysis.

Investigation of three separate nodules of *Brachychiton* exudate indicated that the sample was essentially homogeneous, and contained residues of galactose, rhamnose, and glucuronic acid. Traces of xylose and arabinose were also present in the crude gum, and were detected in large-scale work on the pure polysaccharide. The proportions present, however, are so low they can have very little structural significance. It was noticeable that gum contaminated with bark had more pentoses than did the purer sample. The presence of ash (4·1%) was due at least in part to metal salts of the uronic acid; the aqueous mucilage was neutral to litmus and non-reducing to Fehling's solution. The small amount of methoxyl (1·4%) was present as methyl ester since the purified polysaccharide, isolated after alkaline treatment of the gum, was free from methoxyl groups. The acetyl content (19·3%) resembled that of *Sterculia setigera* [1] and *Cochlospermum gossypium*.[2]

The gum was slightly soluble in water from which it was best precipitated with acetone. Three extracts were obtained of identical composition. Further attempts at fractionation by dissolution of the gum in alkali and precipitation of the " free-acid " form by addition of ethanol were unsuccessful. These results are in harmony with the electrophoretic behaviour of the pure polysaccharide in a borate buffer at pH 10, where a single peak was obtained.

The purified gum, $[\alpha]_D$ +69°, had an equivalent weight of 342 and a uronic anhydride content of 50·1% (calculated for a substance of equivalent weight of 342, 51·5%). The proportion of uronic anhydride is unusually high for a plant gum, but finds parallels in *Sterculia setigera* [1] gum and *Khaya grandfolia* [3] gum. In keeping with this high uronic acid content, and the extreme stability of the glycosiduronic acid linkage, the material was very resistant to hydrolysis. Complete hydrolysis was accompanied by degradation: the solutions invariably darkened during hydrolysis and the yield of hydrolysate was never higher than 80%.

Although a complete quantitative hydrolysis was impossible, estimation [4,5] of the neutral sugars indicated the presence of approximately equal amounts of galactose and rhamnose. The quantity of rhamnose was substantiated by determination [6] of the proportion (24%) of this sugar in the unhydrolysed polysaccharide. A partial hydrolysate (N-sulphuric acid at 100° for 7 hr.), after neutralisation with barium hydroxide, was concentrated to an amorphous solid (A) consisting of neutral sugars and barium uronates (B). The neutral sugars were extracted with methanol and partitioned on a cellulose

column. Crystalline L-rhamnose (3·2% of the original weight of gum) and D-galactose (10%) were separated, and further characterised as crystalline derivatives. On further hydrolysis, the acidic fraction (B) (72% of the original weight of gum) liberated more galactose and rhamnose.

Conversion of the hydrolysate (A), after de-ionisation, into the methyl ester methyl glycoside, reduction with potassium borohydride, and hydrolysis gave a syrup (C) containing galactose, rhamnose, and glucose (paper chromatography), the last being eliminated by treatment of the hydrolysate with D-glucose oxidase. Partition of syrup (C) on a paper column led to the isolation and characterisation of the following sugars in the approximate proportions given: rhamnose (27·5%), galactose (54%), and glucose (18·5%). An alternative method of estimation [4,7] of the proportions of the sugars present in syrup (C) gave rhamnose (24%), galactose (58%), and glucose (17·7%); these figures are in reasonable agreement with the amount of the sugars separated from this syrup. As glucose was not found in any of the gum hydrolysates, it was not present in the original gum, and the glucose isolated from syrup (C) was clearly produced by reduction of glucuronic acid residues by potassium borohydride. Support for this deduction was obtained by the separation and characterisation (as the crystalline *p*-nitroaniline derivative) of glucurone from the acid fraction of the gum hydrolysate.

The presence of D-glucuronic acid in *Brachychiton diversifolium* exudate is in contrast to the positions with the other species of *Sterculia* gums examined, with *Cochlospermum gossypium*, and with *Khaya grandifolia*, all of which contain D-galacturonic acid, although the last of these contains in addition 4-*O*-methyl-D-glucuronic acid. The possible presence of galacturonic acid was considered and the question was raised whether the higher proportion of galactose found in the reduced syrup (C) compared with the amount estimated in the gum hydrolysate might have been produced by reduction of galacturonic acid residues. However, no evidence could be obtained for the presence of this acid in any of the gum hydrolysates by chromatography, by standard colour tests, or by the isolation of mucic acid from the oxidised acid hydrolysate. It might be argued that the inability to isolate free galacturonic acid was due to its degradation under the hydrolytic conditions used since Fischer and Dorfel [8] record 77% and 97% destruction of galacturonic acid by N-hydrochloric acid at 100° in 20 and 40 hours respectively. Against this argument, however, is the fact that the present authors failed to isolate any oligosaccharides or methylated oligosaccharides containing galacturonic acid residues from partial acid hydrolysates of the gum or methylated gum respectively. The present indications are that galacturonic acid residues, if present in the gum, are only there in very small proportion and have escaped detection.

The gum was converted into its fully methylated derivative (OMe, 42·3%) which, like the parent material, was extremely resistant to hydrolysis. Methanolysis with 8% methanolic hydrogen chloride and then with aqueous hydrochloric acid yielded 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (12 parts), 3 : 4-di-*O*-methyl-L-rhamnose (1 part), 2 : 3 : 6-tri-*O*-methyl-D-galactose (10 parts), 3-*O*-methyl-L-rhamnose (4 parts) (characterised as crystalline derivatives), and a mixture of methylated barium uronates (69% of the hydrolysate). The barium uronates were hydrolysed further with 2N-sulphuric acid, and the product was separated into three fractions. Each fraction was converted into the methyl ester methyl glycoside, reduced with lithium aluminium hydride, and hydrolysed. From fraction U1 2 : 3 : 4-tri-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-L-rhamnose were separated. The former was characterised by its analytical constants and as the *N*-phenyl-D-glucosylamine, and the latter by its analytical constants, demethylation, electrophoretic mobility, and oxidation with potassium periodate.[9] As tri-*O*-methylglucose was not present among the neutral sugars isolated on direct hydrolysis of the methylated gum, it must have arisen by reduction of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. The hydrolysates from the second and third fractions both contained 2 : 3 : 4-tri-*O*-methyl-D-glucose, 3-*O*-methyl-L-rhamnose, rhamnose, and a mono-*O*-

methylhexose. In addition, the third fraction contained 2 : 3 : 6-tri-*O*-methylgalactose and a di-*O*-methylhexose (paper chromatography).

The hydrolysate of the methylated, reduced, remethylated polysaccharide was found to contain 2 : 3 : 4 : 6-tetra-*O*-methylglucose in addition to all the methylated derivatives found above (paper chromatography).

Attempted fractionation of the partly hydrolysed acidic material (B) on an ion-exchange resin column gave pure fractions corresponding to glucurone and an aldobiuronic acid. The aldobiuronic acid, $[\alpha]_D$ +63°, gave rhamnose and glucuronic acid (paper chromatography) on hydrolysis and had the correct equivalent weight (332) for an aldobiuronic acid containing these two sugar residues. Reduction with potassium borohydride eliminated rhamnose, indicating that the free reducing group was carried by this sugar. Conversion of the aldobiuronic acid into the methyl ester methyl glycoside, reduction, and remethylation gave a methylated disaccharide which on hydrolysis and separation of the hydrolysate on thick paper gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-L-rhamnose, both sugars being identified by chromatography, ionophoresis and demethylation. A trace of a third component corresponding chromatographically to a tri-*O*-methylhexose was considered to have arisen through under-methylation. These results indicate that the aldobiuronic acid is 2-*O*-α-D-glucuronosyl-L-rhamnose. This is supported by the isolation of 3 : 4-di-*O*-methyl-L-rhamnose from the hydrolysate of the methylated polysaccharide, and of 2 : 3 : 4-tri-*O*-methyl-D-glucose from the reduced methylated uronic acids, and the chromatographic detection of 2 : 3 : 4 : 6-tetra-*O*-methylglucose in the hydrolysate of the methylated, reduced, and remethylated polysaccharide. Although 2-*O*-galacturonosyl-L-rhamnose has been isolated from other plant gums,[2,3,10] and from okra mucilage,[11] this is the first reported isolation of glucuronic acid glycosidically linked to $C_{(2)}$ of L-rhamnose.

It is not possible to formulate a unique molecular structure for the gum. The monomethylrhamnose isolated from the hydrolysate of the methylated polysaccharide and the free rhamnose, and di- and mono-*O*-methylhexoses in the methylated acidic hydrolysate, must have arisen from residues which were triply linked in the gum. It is clear, therefore, that the gum possesses a highly branched structure with galactose and glucuronic acid residues at the ends of the branches. Sugar residues, the presence of which has been definitely established, are:

···Ga 1–···   ···–4 Ga 1–···   ···–2 Rh 1–···   ···–4 Rh 1–···   ···GA 1–···
                                                                  2
                                                                  |

(Ga = D-galactopyranose, Rh = L-rhamnopyranose, GA = D-glucopyruronic acid).

The isolation and characterisation of an aldobiuronic acid indicate that the residue GA 1–2 Rh is also a structural feature of the gum.

Experimental

Analytical Methods.—All solutions were evaporated under reduced pressure below 60°. Unless otherwise stated, hydrolyses were done with 2N-sulphuric acid for 24 hr. at 100°. The cooled hydrolysates were neutralised with barium carbonate, and the filtrates concentrated to syrups. Paper-partition chromatography was carried out on Whatman No. 1 paper at a constant temperature of 20° with the upper layers of the following solvent systems (v/v): (1) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (2) ethyl acetate–pyridine–water (10 : 4 : 3); (3) butan-1-ol–ethanol–water (4 : 1 : 5); (4) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (5) butan-1-ol–acetic acid–water (4 : 1 : 5); (6) benzene–ethanol–water (169 : 47 : 15); (7) ethyl methyl ketone half saturated with water plus ammonia (99 : 1). Papers were sprayed with saturated aqueous solution of aniline oxalate, and 3% *p*-anisidine hydrochloride in butan-1-ol containing a little stannous chloride. R_G, $R_{Gal.}$, and R_F are the rates of travel relative to tetramethylglucose, galacturonic acid, and the solvent front respectively. Electroionophoresis [12] was carried out in borate buffer of pH 10. Whatman 3MM sheets after preliminary extraction with hot benzene–ethanol (1 : 1) were used for the

chromatographic separation of small amounts of material. Specific rotations were measured in water at 18°.

Preliminary Examination of the Gum.—The gum, obtained as light-brown translucent nodules, was contaminated with bark and had a resinous odour. Three separate nodules were powdered, dried to constant weight over phosphoric oxide at 60° (loss 17%), and analysed. They were not significantly different in composition and had ash 4·1; N, 0; OMe, 1·4; AcO, 19·3%. Chromatography of acid hydrolysates (solvent 1) revealed spots corresponding in position and colour with glucurone, rhamnose, galactose, and barium uronates, the last remaining at the starting line.

Purification and Attempted Fractionation of the Material.—(1) Repeated extraction of the crude gum with water and precipitation of the polysaccharide from each extract by the addition of acetone gave identical fractions (paper chromatography of the hydrolysates).

(2) The crude material (4 g.) was dissolved in chilled N-sodium hydroxide (200 c.c.) in an atmosphere of nitrogen. After acidification (pH 2) of the solution with 50% hydrochloric acid, and removal of fragments of bark at the centrifuge, the polysaccharide was precipitated in four fractions by the gradual addition of ethanol (1·3 vols. gave 1·81 g.; 2 vols., 0·30 g.; 5 vols., 0·06 g.; 7 vols., 0·03 g.). Each fraction, freed from chloride ion by trituration with ethanol, was washed with ether and dried over phosphoric oxide (60°/12 mm.) to constant weight. Chromatography of the hydrolysate of each fraction (solvent 1) showed spots identical with those given by the crude gum. Very faint spots corresponding to xylose and arabinose were also detected from the last two fractions.

The recombined fractions were dissolved in water, and the polysaccharide, precipitated with ethanol (3 volumes), was washed and dried as before. It was a white fibrous material (2·2 g.) which had $[\alpha]_D$ +69° (*c* 0·4 in N-NaOH) [Found: equiv., 342 (by titration): uronic anhydride, 50·1 (by decarboxylation); ash, 0·83%; AcO, 0; OMe, 0). A portion (0·05 g.) in borate buffer of pH 10 (10 c.c.) gave a symmetrical peak when examined in the Antweiler micro-electrophoresis apparatus (1·5 mA, 35 V, 10 min.). Autohydrolysis of the polysaccharide for 24 hr. at 100° showed traces of galactose and rhamnose in the concentrated hydrolysate. After hydrolysis with N-sulphuric acid at 100° for 13 hr. ($[\alpha]_D$ +104°, 2 hr. ⟶ +77°, 13 hr.) the solution still contained unhydrolysed polysaccharide. Heating with 2N-sulphuric acid at 100° for 24 hr. was necessary to hydrolyse the polysaccharide completely. The solution then became dark brown and it was necessary to decolorise the neutralised filtrate with charcoal. Quantitative estimation, by the method of Hirst and Jones,[4] of the sugars produced on complete acid hydrolysis of the gum gave, after allowance for a uronic anhydride content of 50%, a 60% recovery of neutral sugars (calc. as percentage of anhydro-sugars in the original gum) comprising 23·5% of rhamnose and 26·5% of galactose. Pridham's colorimetric method [5] gave the proportion of galactose : rhamnose = 1 : 1·09. After allowance for the uronic acid present, this corresponds to 23·8% of anhydrogalactose and 26·0% of anhydrorhamnose in the gum. Colorimetric estimation of rhamnose by the method of Dische and Shettles [6] gave 23·9% of anhydrorhamnose in the purified gum.

Partial Hydrolysis of the Gum and Characterisation of the Neutral Sugars.—The purified gum (9·45 g.) was heated at 100° with N-sulphuric acid (500 c.c.) for 7 hr. The cooled solution was made slightly alkaline with barium hydroxide solution, the excess of alkali being removed immediately with carbon dioxide and the filtrate concentrated to a solid (A) consisting of neutral sugars and barium uronates. The cold aqueous extracts of this material were poured into well-stirred methanol (10 volumes). The precipitated barium salts were exhaustively extracted with hot methanol, and the total methanolic liquors evaporated to a syrup (3·11 g.). The barium uronates (B) (5·38 g.) were left as an amorphous solid. The syrup was fractionated on a cellulose column [13] (85 × 2·7 cm.) with butan-1-ol two-thirds saturated with water as the eluant:

Fraction 1 (1·125—2·175 l.) (0·021 g.), R_F 0·78 (solvent 1), gave a positive Selivanoff reaction.

Fraction 2 (2·325—2·925 l.): L-rhamnose (0·26 g.), R_F variable but identical with that of authentic rhamnose, $[\alpha]_D^{20}$ +8·4° (*c* 1·0). Recrystallisation from butan-1-ol gave L-rhamnose hydrate, m. p. and mixed m. p. 88—89°. The benzoylhydrazone [10] had m. p. and mixed m. p. 186—189°.

Fraction 3 (2·930—6·225 l.) (0·046 g.), R_F 0·34—0·39. Three components were observed in this fraction (paper chromatography) corresponding to rhamnose, xylose, and arabinose. Visual inspection suggested that rhamnose comprised the bulk of the fraction.

Fraction 4 (8·250—12·027 l.): D-galactose (0·951 g.), m. p. and mixed m. p. 163—165°, $[\alpha]_D^{20}$ +83° (*c* 3·5). The diethyl mercaptal [14] had m. p. and mixed m. p. 140—141°.

Fraction 5 (1·43 g.), eluted partly with butan-1-ol–acetic acid–water (4 : 1 : 5) and finally with water, was added to the amorphous barium uronates (B) (total yield 6·81 g.).

Isolation and Characterisation of Glucose after Reduction of the Polysaccharide Hydrolysate.—The mixture of neutral sugars and barium uronates (A) (8·2 g.), after de-ionisation with ion-exchange resin (Amberlite IR-120, H^+ form), was refluxed with 4% methanolic hydrogen chloride for 6 hr. The neutralised filtrate gave on evaporation a solid which was dissolved in water (50 c.c.) and added slowly with stirring to a solution of potassium borohydride (4 g.) in water (60 c.c.). After 2 hr. excess of acetic acid was added and ions were removed on a mixed-bed resin column (Amberlite IR-120 H^+ and IR-4B, OH^-). The eluant was evaporated to dryness, traces of borate being removed by repeated evaporation with methanol. The derived syrup (5·1 g.) was heated at 100° with N-sulphuric acid for 7 hr. Neutralisation of the cooled solution, with barium carbonate, filtration, and evaporation gave a syrup (C) (4·4 g.) containing glucose, galactose, and rhamnose (paper chromatography). Glucose was eliminated on treatment of the syrup (C) (10 mg.) with the specific enzyme D-glucose oxidase. Partition of the syrup (C) (2·19 g.) on a Grycksbo filter paper column (LKB 3391) eluted with butan-1-ol undersaturated with water (prepared by saturating butan-1-ol with water at 0° and allowing the separated organic phase to warm to room temperature) gave:

Fraction (*a*): L-Rhamnose (0·508 g.), characterised as the 2 : 5-dichlorophenylhydrazone, m. p. and mixed m. p. 171°.

Fraction (*b*): D-Glucose (0·150 g.), m. p. and mixed m. p. [after recrystallisation of the isolated material and of authentic D-glucose from ethanol and light petroleum (b. p. 100—120°)] 148—149°, $[\alpha]_D$ +53° (*c* 0·70).

Fraction (*c*): D-Galactose (0·631 g.), characterised as the 2 : 5-dichlorophenylhydrazone, m. p. and mixed m. p. 196—197°. An overlap fraction (0·563 g.) containing galactose and glucose (2 : 1) (paper chromatography, visual estimation) was also separated.

The quantity of glucose in syrup (C) was estimated by determination of the reducing power [15] before and after treatment with D-glucose oxidase [7] (commercial " Dee-O " manufactured by the Takamine Corporation), an enzyme which catalyses the oxidation of glucose to the non-reducing gluconic acid.[16] Synthetic mixtures containing glucose (*a*) 0·25 mg., (*b*) 0·41 mg., galactose (*a*) 1·49 mg., (*b*) 1·30 mg., and rhamnose (*a*) 0·60 mg., (*b*) 0·55 mg. per 5·0 c.c. portion, and the syrup C (2·26 mg. in 5·0 c.c. of water) were each treated with D-glucose oxidase (7·5 mg. in water 1 c.c.) for 3 hr. at 36°. As it was necessary to deproteinise the mixture before measuring the reducing power the mixture was then treated with water (0·40 c.c.), zinc sulphate solution (0·30 c.c.), and barium hydroxide solution (0·30 c.c.) which had been prepared according to Nelson's directions.[17] The precipitate was removed on the centrifuge, a portion of the supernatant solution (5·00 c.c.) withdrawn, and its reducing power estimated. A " blank " determination was carried out simultaneously in the same manner, except that the enzyme was inactivated by heating it at 100° for 10 min. before use. Reducing powers are expressed as c.c. of 0·01N-sodium thiosulphate and in each instance have been calculated so as to refer to a 5 c.c. aliquot part of the original solution:

	(*a*)	(*b*)	C
Reducing power of " blank " (c.c.)	6·55	6·41	5·29
Reducing power of incubated solution (c.c.)	5·65	4·90	3·98
Decrease in reducing power after incubation (c.c.)	0·90	1·51	1·31
Glucose (mg.) per 5·0 c.c.	0·26	0·44	0·39

Hence the syrup (C) contained 17·7% (average of two determinations) of glucose.

Quantitative estimation [4] of the rhamnose and total hexose in syrup (C) gave 24·2 and 75·8% respectively.

Syrup (C), therefore, comprises D-glucose 17·7, L-rhamnose 24·2, and D-galactose 58·1%. The syrup had $[\alpha]_D$ +55° (*c* 1·1). The above composition requires $[\alpha]_D$ +58°.

Examination and Fractionation of the Uronic Acids on Ion-exchange Resin.—The free uronic acids (D) (4·66 g.) obtained by treatment of the barium salts (B) (6·81 g.) with ion-exchange resin (Amberlite IR-120 H^+) had equivalent weight 338. Complete hydrolysis of the acids (D) (20 mg.) liberated more rhamnose and galactose (paper chromatography).

A column of anion-exchange resin (Amberlite IRA-400, acetate form) was prepared,[18] and the uronic acids (D) (3·39 g.) were absorbed on it. After removal of neutral sugars (0·074 g.) by elution with water, the acids were removed with increasing concentrations of aqueous acetic

acid. Only two pure fractions were obtained; four other fractions were isolated but paper chromatography showed them to be mixtures of oligosaccharides which on hydrolysis gave galactose, rhamnose, and glucuronic acid:

Fraction E (0·008 g.), eluted with 2—3% acetic acid, was chromatographically identical with glucurone.

Fraction F (0·355 g.), eluted with 3·5—5% acetic acid, had $[\alpha]_D$ +63° (*c* 1·5), $R_{Gal.}$ 0·24, M_G 0·72 [Found: equiv., 332 (by titration)]. Complete acid-hydrolysis gave rhamnose and glucuronic acid (paper chromatography). Partial hydrolysis, by N-sulphuric acid at 100° for 4 hr., gave rhamnose, glucuronic acid, and unhydrolysed material. Reduction by aqueous sodium borohydride followed by hydrolysis, neutralisation, and de-ionisation gave a product containing only glucuronic acid (paper chromatography). Methylation of Fraction E (0·320 g.) three times with 40% aqueous sodium hydroxide (7·5 c.c.) and dimethyl sulphate (3·0 c.c.) in an atmosphere of nitrogen at room temperature was follwed by two further methylations, one at 50° and another at 70°. After acidification, the solution was extracted with cold chloroform. Removal of the chloroform gave a syrup (0·148 g.) which was methylated with methyl iodide (2·3 c.c.) and silver oxide (1·2 g.) under reflux. The product (0·140 g.), dissolved in dry methylal (2·0 c.c.), was reduced with lithium aluminium hydride (0·1 g. in 1 c.c. of methylal). Excess of reagent was destroyed by cautious addition of water and the product (0·098 g.) isolated by methylal-extraction. Two further methylations with Purdie's reagents gave a syrup (0·088 g.) which was hydrolysed by N-sulphuric acid at 100° for 6 hr., and the product (0·060 g.) was separated on thick paper with solvent (3). The first component was identical with 2 : 3 : 4 : 6-tetra-*O*-methylglucose chromatographically and ionophoretically. Demethylation gave glucose. The second component was identical with 3 : 4-di-*O*-methylrhamnose chromatographically and ionophoretically (M_G 0·36). Demethylation gave rhamnose. A very small quantity of a third component (R_G 0·76) gave glucose on demethylation.

Isolation of Glucurone after Complete Hydrolysis of the Polysaccharide.—Free uronic acids (0·20 g.) isolated as before from a completely hydrolysed sample of the gum were separated on sheets of thick paper (solvent 4) into two fractions:

Fraction 1 (0·039 g.) was chromatographically identical with glucurone. The derived yellow crystalline *p*-nitroaniline derivative [19] (0·020 g.) had m. p. and mixed m. p. 128—130° (after recrystallisation from methanol).

Fraction 2 (0·116 g.) had $[\alpha]_D$ +33·6° (*c* 1·16) and gave a single spot on chromatography identical with glucuronic and/or galacturonic acid. Treatment with basic lead acetate [20] gave a yellow-brown precipitate similar to that given by authentic glucuronic acid and distinct from the brick-red precipitate given by a control sample of galacturonic acid. A portion (0·10 g.) in water was oxidised with bromine (1 c.c.) for 4 days at 30—35° (nitric acid oxidation was avoided because it would convert any trace of galactose, which might have persisted in this fraction, into mucic acid). The residue obtained after removal of bromine by aeration and evaporation of the solution to dryness was dissolved in N-sodium hydroxide. Filtration followed by acidification of the filtrate by dropwise addition of hydrochloric acid failed to yield mucic acid even on prolonged storage.

Methylation of the Polysaccharide.—The pure polysaccharide (22 g.) was dissolved in 30% sodium hydroxide solution (600 c.c.) in an atmosphere of nitrogen which was maintained during all further methylations involving sodium hydroxide. The solution was cooled to 10° and dimethyl sulphate (270 c.c.) was added during 8 hr. with vigorous stirring, the temperature being kept below 15°. The mixture was set aside for 18 hr., after which the methylation was repeated twice. After cooling to 5° the solution was acidified by cautious addition of 50% sulphuric acid, and the mixture dialysed against running water for 24 hr. Concentration to 200 c.c. was followed by three methylations as before, acidification, and dialysis for 4 days. The pH was adjusted to 2 with sulphuric acid and a further equal volume of acid added. Dialysis (until the solution was free from sulphate ions) and evaporation gave an amorphous solid (14·0 g.) (Found: ash, 5·0; OMe, 28·4%). A portion (9·0 g.) of this material, dissolved in water, was de-ionised with Amberlite IR-120 H^+ resin and then stirred overnight with an 8—10-fold excess of silver carbonate. Filtration and freeze-drying gave an amorphous silver salt which was triturated with methyl iodide (50 ml.); the mixture was then refluxed with the addition of silver oxide (4 g.) in portions (0·5 g.). The product obtained on filtration and evaporation was methylated three times according to Purdie's directions. The fully methylated polysaccharide (6·2 g.) had $[\alpha]_D^{18}$ +68·4° (*c* 1·17 in $CHCl_3$) (Found: OMe, 42·3%).

Hydrolysis of the Methylated Polysaccharide.—Methanolysis of the polysaccharide (6·0 g.)

with 8% methanolic hydrogen chloride (150 c.c.) at 100° in a sealed tube for 18 hr. was followed by hydrolysis with 4% hydrochloric acid for 6 hr. at 100°. Neutralisation with silver carbonate and removal of silver ions with hydrogen sulphide gave a solution which was made alkaline with barium hydroxide, excess of alkali being then removed by passage of carbon dioxide through the solution. A viscous syrup (5·73 g.) was isolated from which the neutral methylated sugars were extracted by refluxing dry diethyl ether (4 × 100 c.c.). Removal of the ether gave a syrup (1·54 g.). The residual barium salts (4·0 g.) were further hydrolysed with 2N-sulphuric acid at 100° for 21 hr. A brown amorphous solid was obtained which gave a further quantity of neutral sugars (0·20 g.) on extraction with dry ether. The residue (2·10 g.) consisted of methylated barium uronates.

Fractionation of the Neutral Methylated Sugars.—The methylated sugars (1·74 g.) were partitioned on a cellulose column (850 × 30 cm.), eluted with butan-1-ol–light petroleum (b. p. 100—120°) (30 : 70; v/v) saturated with water. After the collection of 2·3 l. the proportions in the eluant were changed to (60 : 40; v/v) and after 12 l. had been collected the eluant was changed to water. Fractions were as follows, R_G values being for solvent (3):

Fraction G (1·768—1·92 l.): Syrupy 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (0·127 g.), R_G 0·88, $[\alpha]_D$ +113° (*c* 1·14) (Found: OMe, 51·9. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The derived anilide had m. p. 190—191°, $[\alpha]_D^{18}$ −69° ⟶ +35° (*c* 0·75 in acetone).

Fraction H (1·928—2·230 l.): A syrup (0·307 g.), R_G 0·86. Electroionophoresis showed two components of M_G 0·36 and 0·0 respectively. Separation was achieved by partition on a column of " Celite 535 " (50 × 3·0 cm.) developed with solvent (6). The syrup (0·305 g.), dissolved in the organic phase of solvent (6) (3 ml.), was absorbed on dry Celite (3 g.), and the mixture was packed on top of the column, giving:

Fraction (i) (775 c.c.—1025 c.c.), syrupy 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (0·214 g.), R_G 0·88, $[\alpha]_D^{18}$ +112° (*c* 2·1) (Found: OMe, 52·0%).

Fraction (ii) (1275—1300 c.c.), crystalline 3 : 4-di-*O*-methyl-L-rhamnose (0·036 g.), R_G 0·85, M_G 0·36, m. p. 93—94° $[\alpha]_D^{18}$ +23° (*c* 2·3) (Found: OMe, 32·9. Calc. for $C_8H_{16}O_5$: OMe, 32·3%) [demethylation gave rhamnose (paper chromatography)].

Fraction J (4·96—6·84 l.): Syrupy 2 : 3 : 6-tri-*O*-methyl-D-galactose (0·436 g.), R_G 0·71, $[\alpha]_D^{20}$ +96° (*c* 1·8) (Found: OMe, 40·9. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The derived lactone had m. p. 97—99°, $[\alpha]_D^{18}$ −40° ⟶ −30° (*c* 1·2) (constant), and gave one component on chromatography in solvents (4) or (5) (detected by spraying with alkaline hydroxylamine hydrochloride, then ferric chloride [21]).

Fraction K (10·8—11·84 l.): Crystalline 3-*O*-methyl-L-rhamnose (0·140 g.), R_G 0·56, m. p. and mixed m. p. 114—115°, $[\alpha]_D$ +30° (*c* 1·0) (Found: OMe, 17·0. Calc. for $C_7H_{14}O_5$: OMe, 17·4%). The *X*-ray powder photograph was identical with that of the authentic material and distinct from that of 4-*O*-methyl-L-rhamnose. The derived lactone had $[\alpha]_D^{15}$ −20° (*c* 1·0).

Fraction L: Water-washings; methylated barium uronates (0·24 g.).

Examination of the Methylated Barium Uronates.—The combined methylated barium uronates (2·34 g.) were converted into the free uronic acids (1·70 g.) by treatment with Amberlite IR-120 H^+ resin. Partition on " Celite " as for fraction H with butan-1-ol–*n*-butyl acetate–acetic acid–water (50 : 6 : 4 : 40) as eluant gave three fractions:

(*a*) Fraction U 1 (0·35—0·65 l.): A syrup (1·0 g.), $[\alpha]_D$ +59° (*c* 0·93). Paper chromatography showed a single spot (solvent 4) identical with that of 2 : 3 : 4-tri-*O*-methylglucuronic acid, but development of a paper chromatogram with solvent 7 revealed two spots. Conversion of a portion into the methyl ester glycoside by treatment with 3% methanolic hydrogen chloride was followed by dissolution in dry tetrahydrofuran (5 c.c.) and reduction by the dropwise addition of a saturated solution of lithium aluminium hydride in dry tetrahydrofuran. The solution was refluxed gently for 30 min., then cooled and the excess of hydride was destroyed by ethyl acetate. Addition of water, evaporation to dryness, extraction of the residues with boiling acetone, then with dry chloroform, and evaporation of the extracts gave a syrup. Hydrolysis with N-hydrochloric acid (5 c.c.) at 100° for 6 hr. gave a syrup (0·279 g.) which was partitioned on a cellulose column (25 × 42 cm.) with solvent 7. Solvent was removed from the respective fractions at 30—40° in a stream of nitrogen.

Fraction M (0·054 g.) was purified by separation on Whatman 3MM paper with solvent 7. A syrup (0·028 g.) was isolated which had R_G 0·88 (solvent 3), R_F 0·83 (solvent 7), M_G 0·36, $[\alpha]_D$ +17° (*c* 0·36) (Found: OMe, 29·5. Calc. for $C_8H_{16}O_5$: OMe, 32·3%). Demethylation of a portion of the syrup gave rhamnose (paper chromatography). The syrup was oxidised in aqueous solution with potassium periodate.[9]

Fraction *N*: A syrupy mixture (0·137 g.) of fractions M and P (paper chromatography, solvent 7).

Fraction *P*: 2 : 3 : 4-Tri-*O*-methyl-D glucose (0·062 g.), $[\alpha]_D$ +65° (*c* 0·18),[22] R_G 0·85 (solvent 3), R_F 0·72 (solvent 7), M_G 0·0 (Found: OMe, 41·3. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The derived *N*-phenyl-D-glucosylamine had m. p. and mixed m. p. with 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine 145—146°.[22]

(*b*) Fraction U 2 (0·85—1·6 l.) (0·239 g.): This fraction was contaminated with neutral sugars from which it was separated by elution on Whatman 3MM paper with solvent 7. The uronic acid remained on the starting line and was eluted with water, de-ionised (Amberlite IR-120 H^+), and evaporated to a syrup (0·083 g.) which had $[\alpha]_D$ +80° (*c* 0·33). A portion (0·060 g.) was converted into the ester glycoside, reduced, and hydrolysed as before. Chromatography (solvent 3) showed 2 : 3 : 4-tri-*O*-methyl-D-glucose R_G 0·85, 3-*O*-methyl-L-rhamnose R_G 0·56, rhamnose R_G 0·28, and a mono-*O*-methylhexose R_G 0·23. The material of R_G 0·85 was separated on Whatman 3MM paper and examined electroionophoretically: a single spot of M_G 0·0 was detected.

(*c*) Fraction U 3 (1·625—2 l.) (0·10 g.) had $[\alpha]_D^{20}$ +93° (*c* 0·38). Treatment as for the previous fraction gave a syrup containing six components (paper chromatography, solvents 3 and 7). These included the four sugars present in the previous fraction, together with 2 : 3 : 6-tri-*O*-methyl-D-galactose R_G 0·71 and a di-*O*-methylhexose R_G 0·46.

Reduction and Remethylation of the Methylated Polysaccharide.—The methylated gum (OMe, 42·3%) (0·75 g.) in dry tetrahydrofuran (1·5 c.c.) was reduced with lithium aluminium hydride as for fraction U 1. The solid product (0·61 g.) had $[\alpha]_D$ +75° (*c* 1·03 in $CHCl_3$). This material (0·41 g.) after four methylations with Purdie reagents had $[\alpha]_D$ +45·8° (*c* 2·6 in $CHCl_3$) (Found: OMe, 41·8%). Methanolysis with 4% methanolic hydrogen chloride for 8 hr. under reflux, followed by hydrolysis with N-hydrochloric acid for 16 hr. at 100°, gave a syrup (0·238 g.). Chromatography (solvents 3 and 7) showed all the components of fraction U 2 except the 2 : 3 : 4-tri-*O*-methylglucose and in addition 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose R_G 1·0, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose and 3 : 4-di-*O*-methylrhamnose R_G 0·85. Tetramethylgalactose has R_F 0·92 in solvent 7.

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BARRY DEGRADATION OF LAMINARIN

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Recent work on insoluble laminarin has revealed the presence of some β-1:6-glucosidic linkages.[1,2] The molecule therefore may consist of unbranched chains of β-1:3-linked glucose units occasionally interrupted by β-1:6-links or it may be branched. In the absence of positive evidence for branching some workers[1] prefer to leave the question open. By contrast others[2] are of the opinion that the molecule is slightly branched with β-1:3-linked chains interlinked by β-1:6-glucosidic linkages, since 80% of the molecules (by weight) have a degree of polymerization of 60 and an average chain length of 23. Subjection of laminarin to successive oxidation with sodium metaperiodate and degradation with phenylhydrazine[3] gives, after three oxidations and two degradations a residual oxopolysaccharide (D_2O_3), the molecules of which are sufficiently large to be retained inside a dialysis sac, and which retain the characteristic property, of insoluble laminarin, of slow precipitation from aqueous solution.

The oxopolysaccharide is isolated at each stage by precipitation with alcohol after dialysis against running water (3 days). Degradation with phenylhydrazine is followed by exhaustive extraction with ether and isolation of the degraded polysaccharide (OD) by freeze-drying the aqueous solution. The yield and nitrogen contents of the materials isolated are given in the Table.

Table

	Weight (g.)	*Yield* (%)	*N content* (%)
Laminarin	4·16		
Oxopolysaccharide (O_1)	3·62	87	
Degraded oxopolysaccharide (O_1D_1)	2·86	79	0·94
Degraded oxopolysaccharide (D_1O_2)	2.29	80	0·51
Degraded oxopolysaccharide (O_2D_2)	2·00	87	1·01
Degraded oxopolysaccharide (D_2O_3)	1·52	76	0·48

The small loss observed during dialysis of O_1 is in accord with the postulated heterogeneity of the laminarin sample.

Periodate oxidation and phenylhydrazine degradation only attacks and removes the residues at either end of a 1:3-linked chain, but wherever 1:6-links occur the molecule is cleaved. Their presence at intervals along an unbranched molecule of average chain length of 23 would, under this treatment, give rise to small fragments of dialysable size. However, if the 1:6-links occur only as inter-chain linkages then chains of approximately the same length would remain after oxidation and degradation. Unless therefore the 1:6-links occur exclusively near the ends of the chains, the results now recorded support the idea of a branched structure for the laminarin molecule.

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THE PRESENCE OF L-GULURONIC ACID RESIDUES IN ALGINIC ACID

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The presence of L-guluronic acid residues in alginic acid has been reported by Fischer and Dörfel.[1] This has been confirmed by recent work in this Department. Paper chromatography of the syrup obtained after hydrolysis of alginic acid showed four spots corresponding to gulurone, mannurone, guluronic acid and/or mannuronic acid and partially hydrolyzed material. Separation of this syrup on a cellulose column, followed by glycosidation and reduction of the two fractions containing respectively the lactones and the mono-uronic acids gave syrups, each of which on hydrolysis contained only mannose and gulose (paper chromatography and ionophoresis). Although it is difficult to distinguish between mannose and gulose by paper chromatography, ionophoresis in borate buffer (pH 10) for 5 hours at 750 volts gives two well-defined spots for these two sugars with M_G 0·46 and 0·59 respectively. Although small variations in M_G were obtained on changing the conditions of ionophoresis of the hydrolysates the spots obtained were always identical with mannose and gulose run as controls. It is considered that the gulose can only have arisen by reduction of guluronic acid residues present in the parent molecule.

If alginic acid consisted entirely of 1:4-linked mannuronic acid residues then periodate oxidation followed by bromine oxidation and hydrolysis would result in a mixture of glyoxylic and *meso*tartaric acids with zero rotation. 1:4-Linked L-guluronic acid residues, if present, would, however, give rise to L(+)tartaric acid and not the *meso* acid and the final mixture would have a positive rotation. We have found that the polymeric material isolated after periodate and bromine oxidation of alginic acid had $[\alpha]_D$-31° (H_2O), and that hydrolysis of this material caused the rotation to change from α_D-0·19° (initial)→ -0·03 (4 hours) → ±0° (14 hr.)→+0·11° (22 hours, const.), indicating the presence of L(+) tartaric acid. The possibility that the positive rotation was due to a small quantity of mannurone which had arisen from unoxidized mannuronic acid residues was considered. Paper chromatography of the hydrolysate in an acid eluant gave a spot identical with tartaric acid. Ionophoresis (borate buffer, pH 10) showed the absence of any mannurone. Furthermore, the addition of crystalline ammonium molybdate to a portion of the hydrolysate greatly enhanced the rotation, a property characteristic of L(+)tartaric acid.[2] Addition of an equivalent quantity of ammonium molybdate to an aqueous solution of mannurone reduced the rotation to a negative value. Hence these results have provided additional evidence for the presence of L-guluronic acid residues in alginic acid. This work is proceeding and will be published in more detail later.

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THE POLYSACCHARIDES OF ACROSIPHONIA CENTRALIS (SPONGOMORPHA ARCTA)

By

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A comparative survey of the polysaccharides from green seaweeds is now in progress. Following work on the water soluble polysaccharide from *Cladophora rupestris*, an investigation on similar material from *Acrosiphonia centralis* (*Spongomorpha arcta*), the weed most closely related botanically to *C. rupestris*, is now reported. These two weeds are so similar morphologically that collection of pure speciments of *Acrosiphonia* is difficult and we are grateful to Dr. M. de Valera and to Mr. H. T. Powell (Millport) for supplies of this weed.

A hot water extract (8% of the dried weed) from the Millport sample contained a complex sulphated polyuronide (SO_4'' *ca.* 7%, uronic anhydride 20%) which gave, on partial hydrolysis, glucose, xylose and rhamnose in the molecular proportions of 10 : 17 : 13 and trace quantities of galactose and mannose. In addition, 45% of the hydrolysate consisted of oligouronic acids composed mainly of rhamnose and glucuronic acid. A small quantity of a glucose-rich acetate, free from uronic acid and sulphate residues, was separated from the acetylated extract. However attempts to fractionate the unacetylated material or its methyl ether into more that a single entity were unsuccessful.

Methylation and periodate oxidation studies indicated the presence of X1 . . . . . .4X1 . . . . . .4G1 . . . . . .4G1 . . . (G=glucose, 6
X = xylose) linkages in the main fraction, and G1 . . . . .4G1 . . . . . 4G1 . . . in the glucose rich fraction.
6

A comparison will be drawn between this material and the polysaccharides from *C. rupestris* and *Ulva lactuca*. *C. rupestris* differs from *A. centralis* in that the weed residue of the former contains a glucan resembling Floridean starch.

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ANALYSIS OF THE CARBOHYDRATES OF *CLADOSTEPHUS* SP.

By R. S. FANSHAWE and ELIZABETH PERCIVAL

Cladostephus verticillatus, a brown seaweed belonging to the genus *Cladostephaceae*, was collected from Nobska Point, La Jolla, California, during 1955 and air-dried. Alcoholic, aqueous, and sodium carbonate extracts have been analysed. The major carbohydrates are alginic acid, laminarin, fucoidin and mannitol. Quantitative estimations of the fucoidin and laminarin content (2% and 20% of dried weed, respectively) of *Cladostephus spongiosus* collected near Plymouth have also been carried out.

Experimental

All analyses were carried out on dried and milled samples. All solutions were concentrated under reduced pressure at 36°. Paper chromatograms were eluted with (*a*) ethyl acetate : acetic acid : water (3 : 1 : 3, v/v) and (*b*) benzene : butanol : pyridine : water (1 : 5 : 3 : 3, v/v).

Cladostephus verticillatus

Ash.—The sample was ignited at a dull red heat in a platinum crucible, cooled and a few drops of concentrated nitric acid added, the crucible reheated, cooled, a few drops of concentrated sulphuric acid added, and the crucible reheated to constant weight.

Nitrogen.—Nitrogen was determined by the Kjeldahl method in a micro-apparatus.

Mannitol.—Mannitol in the weed was estimated by the method of Cameron *et al.*[1]

Alcohol-soluble carbohydrate.—The weed was extracted with boiling 80% aqueous ethanol for 2 hours (twice). The combined extracts were concentrated and the solution clarified.[2] Inorganic ions were removed by ion-exchange electrodialysis.[3] The ion-free solution, on concentration to small volume, deposited crystals. After removal of the crystals the residual solution was analysed chromatographically with eluant (*a*) and *p*-anisidine and aniline oxalate sprays.

Water-soluble carbohydrates.—The alcohol-extracted weed was stirred with cold water for 8 hours. Removal of the weed gave a clear solution which was set aside for 48 hours. A white precipitate (A) was deposited which caused no colour change on addition to a dilute solution of iodine. The residual solution was concentrated to small volume (150 ml.) and added with stirring to ethanol (450 ml.). The resulting precipitate, after filtration, was dissolved in water, dialysed against running water (100 hours) and reprecipitated into ethanol. The precipitate (B) was filtered and dried (P_2O_5) under high vacuum. The residual weed was extracted twice with boiling water for 8 hours. The combined extracts were concentrated and the solution poured into ethanol. The resulting precipitate (C) was filtered and dried.

Sodium-carbonate-soluble carbohydrate.—The residual hot-water-extracted weed was kept under sodium carbonate solution (3%) for 2 hours at 50° and thereafter for 15 hours at room temperature. Removal of the weed and addition of calcium chloride (10%) to the solution gave a light brown precipitate which was removed by centrifuging. Addition of N-hydrochloric acid to the precipitate until the solution was acid to litmus left a brown precipitate (D) which was washed with N-hydrochloric acid to remove Ca^{2+} and then with ethanol until Cl'-free, and dried (P_2O_5).

Alginic acid.—After extraction with alcohol and water, the residual weed was dried (98°) and a portion (2·31 g.) analysed quantitatively for alginic acid.[1]

Reducing sugars.—The precipitates (A), (B), (C) and (D) were hydrolysed in sealed tubes with 2N-sulphuric acid (4N for D) at 95° for 10 hours, cooled, neutralized with barium carbonate, filtered and washed. The filtrate and washings were concentrated to small volume, deionized, and the solutions analysed chromatographically. The papers were developed with solvent (*b*) for 40 hours, dried and sprayed with aniline oxalate, *p*-anisidine hydrochloride and ninhydrin. The relative proportions of the respective sugars in the hydrolysate from (B) were determined by separation on paper and estimation by the Somogyi method.[4]

Cladostephus spongiosus

Fucoidin.—Fucoidin in the weed was determined by hydrolysis and conversion to fucose methylphenylhydrazone.[5]

Glucan.—The alcohol-extracted weed was further extracted for 6 hours with cold water. After removal of the residual weed an aliquot (5 ml.) of the solution with sodium acetate–acetic acid buffer (pH, 5 ; 1·0 ml.) was incubated at 37° with barley β-glucosidase preparation (supplied by Dr. D. J. Manners). A ' blank ' containing enzyme, buffer and distilled water was incubated simultaneously. The solution and ' blank ' were each analysed by paper chromatography [eluant (*a*), aniline oxalate spray] after incubation for 0·5, 1·5 and 3 hours.

The glucan content was determined by a modification of the method used by Cameron *et al.*[1] for the estimation of laminarin. A specimen of notatin (glucose oxidase), supplied by Dr. D. J. Manners, was used instead of the yeast suspension. The reducing power of the seaweed hydrolysate (5 ml.) was determined before and after treatment with glucose oxidase, an enzyme which catalyses the oxidation of glucose to the non-reducing gluconic acid.[6] An aqueous preparation (1·0 ml.) of glucose oxidase was added to a solution (5·0 ml.) of the seaweed hydrolysate. The mixture was incubated[7] for 3 hours at 36° and then deproteinized by the addition of water (0·40 ml.), zinc sulphate solution and barium hydroxide solution (0·30 ml. each) which had been prepared according to Nelson.[8] The precipitate was removed on the centrifuge and the supernatant solution (5·0 ml.) withdrawn and the reducing power estimated. A ' blank ' determination was carried out simultaneously in exactly the same manner except that the enzyme preparation was inactivated by heating at 100° for 10 minutes before use.

Results

The alcoholic extract was shown chromatographically to consist mainly of mannitol with trace quantities of sucrose, galactose, mannose and an unidentified spot R_G 0·57. Mannitol (1·8% of the dried weed[1]) was isolated and had m.p. and mixed m.p. with an authentic specimen 163°. The derived hexa-acetate[9] had m.p. 122–123°.

Chromatographic analysis of the hydrolysate from precipitate (A) showed a single spot which moved at the same rate as glucose run as a control. The aqueous extract from *Cladostephus spongiosus*, after incubation with β-glucosidase at 37°, showed spots corresponding to glucose together with slower-moving oligosaccharides after 0·5, 1·5 and 3 hours' incubation. The 'blank' solution gave no spots. Quantitative estimation gave 20% glucan in the dried weed.

Precipitate (B) (0·17 g. from 10 g. dried weed) had ash (sulphated) 18% (containing Mg^{2+}, Fe^{3+}, Na^{+} and K^{+}), and N 1·8%. Examination of the hydrolysate on a paper chromatogram [eluate (*b*) (40 h.)] showed spots corresponding to glucose, galactose, mannose, xylose and trace quantities of rhamnose and fucose. The latter sugar probably came from hydrolysed fucoidin. Amino-acids were detected with ninhydrin spray. Quantitative estimation of the relative proportions of glucose : galactose : mannose : xylose in the hydrolysate from (B) gave 1·1 : 2·8 : 1·0 : 1·0.

Precipitate (C) (0·3 g. from 10 g. dried weed) had ash (sulphated) 26·3% (containing Ca^{2+}, Fe^{3+}, Mg^{2+}) and N 1·8%. Paper chromatographic analysis of the hydrolysate showed the same sugars as the hydrolysate from (B) in, as far as could be judged visually, the same relative proportions.

The hydrolysate from (D) gave a single spot on chromatographic analysis with eluate (*a*). This was identical with mannuronic acid run as control. Estimation of alginic acid on the dried weed residue gave 25% (11·5% of dried weed).

The fucoidin content of *Cladostephus spongiosus* was equal to 2% of the dried weed.

Conclusion

The glucan has all the properties of laminarin since it gave no colour with iodine, was precipitated from aqueous solution on standing and was hydrolysed by β-glucosidase to glucose. The principal carbohydrates of *Cladostephus* sp. are therefore alginic acid, laminarin, fucoidin and mannitol which agrees with the general pattern of carbohydrate constituents of the brown seaweeds.[10] The presence of a relatively small quantity of other water-soluble polysaccharides made up of galactose, xylose and mannose units is in agreement with the results of Jensen.[11] This author hydrolysed twelve other species of the common brown algae and found these three sugars (paper chromatography) in the hydrolysates.

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227. *The Constitution of Xylan from the Green Seaweed* Caulerpa filiformis.

By I. M. Mackie and Elizabeth Percival

After alcoholic and aqueous extraction of *Caulerpa filiformis*, dilute alkaline extraction of the residual weed affords a xylan. Methylation and other studies provide evidence for a structure comprising chains of β-D-xylopyranose residues linked between $C_{(1)}$ and $C_{(3)}$.

Comparatively little is known about the polysaccharides of the green seaweeds. Water-soluble materials from *Cladophora rupestris* [1] and from *Ulva lactuca* [2] consist of complex polysaccharide material containing a wide variety of sugar residues. In spite of repeated fractionation with various reagents, no separation of these materials was achieved. Chloroform extraction of acetylated cladophoran did, however, give a small yield of a glucose-rich fraction. Preston and his colleagues,[3] examining the cell-wall material of a number of green seaweeds by *X*-ray and hydrolytic methods, have also revealed the complexity of their polysaccharide constituents.

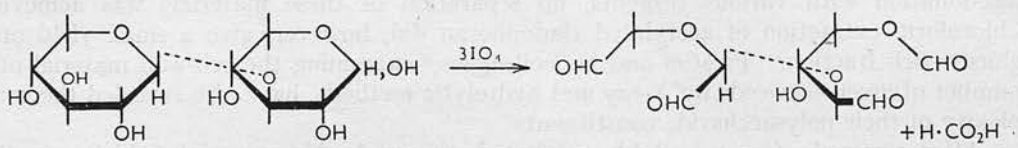
After removal of water-soluble sulphated polysaccharide material (which contains glucose, galactose, mannose, xylose, rhamnose, and uronic acid residues) from the green seaweed, *Caulerpa filiformis*, mild treatment of the insoluble residue with chlorite [4] was followed by extraction with dilute sodium hydroxide solution at room temperature. Acidification of the alkaline extract afforded a crude xylan (*ca.* 5·0% of the dried weight of weed) (Ash, 2·0; SO_4^{2-}, 3·6%) comprising xylose (90%) and glucose (10%). Precipitation with various copper salts [1,5,6] or with Cetavlon [7] failed to reduce the glucose content, but extraction with water and dialysis afforded xylan (B) (4·5%) containing *ca.* 4% of glucose (ash, 0·5%; SO_4^{2-}, nil). A pure xylan (C) (3·3% dried weight of weed) devoid of glucose and sulphate residues was isolated after exhaustive extraction of material (B) with water. In view of the limited quantity of weed available, and the loss of xylan during complete purification, large-scale investigations were carried out on polysaccharide (B). The extraction of the weed and its treatment with chlorite were carried out under mild conditions in order to minimise the possible degradation of the xylan. During the purification no obvious signs of degradation were apparent; in particular the specific viscosities and periodate consumption of xylan (B) and xylan (C) were similar.

Xylan (B) afforded 92% of crystalline D-xylose on hydrolysis. By two series of five methylations, each with methyl sulphate and sodium hydroxide in an atmosphere of nitrogen,[5] a methylated xylan was prepared containing 31·5% of methoxyl. Four treatments with Purdie's reagents gave a product containing 37·2% of methoxyl. Fractionation using chloroform–light petroleum separated a negligible quantity of lower methylated material (OMe, 22%). The major fractions (OMe, 37—37·4%) were recombined and methylated with sodium and methyl iodide in liquid ammonia.[8] The isolated material had $[\alpha]_D^{18}$ −60° and contained 37·8% of methoxyl (Calc. for $C_7H_{14}O_4$: OMe, 38·7%). Repeated methylation and dialysis had not apparently degraded the xylan seriously, since a xylan acetate prepared by mild acetylation had a specific viscosity of the same order as had the methylated xylan measured under identical conditions.

The methylated xylan was hydrolysed with methanolic hydrogen chloride giving the glycosides, and from them the methylated sugars were obtained by hydrolysis with dilute hydrochloric acid. Separation on a cellulose column gave the following molar percentage separation of methylated xyloses: 2 : 3 : 4-tri-*O*-methylxylose (2·9), 2 : 4-di-*O*-methylxylose (95·2) and a mixture of 2- and 4-monomethylxylose (1·9). Both the tri- and the di-*O*-methyl sugars were crystalline. The amount of trimethylxylose corresponds to one non-reducing end group for every 34 xylose residues, but this figure can only be regarded as very approximate as the weight of each fraction was recorded after extensive purification. The value of one non-reducing end group for every 47 anhydro-xylose units (average of two experiments), determined on the methylated hydrolysate by hypoiodite

oxidation after separation on a paper chromatogram,[5] is probably a more accurate figure. The presence of monomethylxylose (*ca.* 1·9%) is easily accounted for on the grounds of under-methylation, and of demethylation during hydrolysis. Taken as a whole the methylation results show that the xylan is made up of linear chains of 1 : 3-linked β-D-xylopyranose residues, and that the majority, at least, of the chains are unbranched; the β-configuration being inferred from the negative rotation.

Periodate-oxidation studies were undertaken to obtain further evidence on the constitution of this polymer. A linear 1 : 3-linked xylan, with a reducing group at one end of the molecule, would be attacked by periodate only at the ends of the chains with the reduction of 3 mols. of periodate and the formation of 1 mol. of formic acid and a stable formyl ester, but no formaldehyde.[9] On this assumption the consumption of one mol. of



periodate for about 14 xylose units gives an average chain length of 42, and the yield of formic acid gives a similar figure, since about 1 mol. of formic acid is liberated from 43 xylose units. The small yield of formaldehyde may be due to a small amount of hydrolysis of the formyl ester during the oxidation. On the other hand it is very probable that during the extraction of the xylan with alkali some modification of the reducing group takes place. In the absence of definite knowledge regarding the nature of this group the interpretation of the periodate oxidation results can only be regarded as tentative. The liberation of free xylose (90%) on acid hydrolysis of the oxopolysaccharide does, however, provide further evidence for a 1 : 3-xylosidic linkage.

The contaminating glucose in xylan (B) was separated from the methylated hydrolysate as 2 : 3 : 4 : 6-tetra-*O*-methylglucose (trace, paper chromatography), crystalline 2 : 4 : 6-tri-*O*-methylglucose, and a small quantity of 4 : 6-di-*O*-methylglucose. Taken in conjunction with the small change in rotation of the xylan, on removal of this glucan, all the evidence points to this polymer's being of the laminarin type with 1 : 3-β-linked glucopyranose units. The periodate consumption of such a molecule would be of the same order as that of the xylan, since again only the end residues are liable to attack by this reagent. Evidence for this is the very similar uptake of periodate by xylan (B) (containing 4% of glucose) and by xylan (C) which is glucose-free.

Bearing in mind all the results it seems justifiable to assign to the xylan from *Caulerpa filiformis* a structure containing linear chains of D-xylopyranose units joined by 1 : 3-β-linkages.

Although xylose is a constituent of the polysaccharides of many green seaweeds [3] this is the first time a pure xylan has been isolated from this source. 1 : 4-β-Linked xylans constitute the major polysaccharide of the hemicelluloses of land plants and a xylan containing 80% of 1 : 4-linked and 20% of 1 : 3-linked β-D-xylopyranose units has been separated from the red seaweed, *Rhodymenia palmata*.[11] The present xylan is unique in consisting solely of 1 : 3-linked residues.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper. Paper ionophoresis was in borate buffer (pH 10) at 750 v for 5 hr. Evaporations were carried out at 40°/15 mm.

The green seaweed, *Caulerpa filiformis*, collected from rock pools near Cape Town in February, 1957, was kindly made available to us by Dr. A. M. Stephen and Mr. R. H. Simons. The weed was dried at 60° and ground to a pale green powder. Extraction of this dried powder (15 g.) with 80% aqueous alcohol under reflux removed most of the colouring matter. Chromatographic examination of the alcoholic extract, after deproteinisation [12] and deionisation,[13]

indicated the presence of sucrose and glucose. The residual weed was extracted with dilute hydrochloric acid (pH 3—4) at 70° for 6 hr. (twice) and the combined extracts, after dialysis and freeze-drying, gave an off-white powder (Found: N, 2·5%). Subsequent extracts were therefore treated with trichloroacetic acid before dialysis. After several days the precipitated protein was removed on the centrifuge and the supernatant liquid dialysed against running water for several days. Concentration of the dialysed solution followed by freeze-drying gave an off-white powder (1·5 g.), $[\alpha]_D$ +120° (*c* 3·0 in water) [Ash (direct), 13·5; (as sulphate), 15·4; SO_4^{2-} in ash, 3·8% of dry weight of polysaccharide; total SO_4^{2-}, 7·5; uronic anhydride, *ca.* 8; N, 1·5%].

Chromatography of the hydrolysate (N-sulphuric acid at 100° for 4 hr.) by the method of Flood, Hirst, and Jones [14] (*a*) and of Pridham [15] (*b*) showed that the polysaccharide material contained the following approximate molar proportions of the sugars: galactose (*a*) 4, (*b*) 4; glucose (*a*) 14, (*b*) 15; mannose (*a*) 2, (*b*) 2; xylose (*a*) 2, (*b*) 1; and rhamnose (*a*) 0·5, (*b*) 0·2. The presence of anhydro- and/or ketose sugar residues in the hydrolysate and in the polysaccharide was indicated by the red colour given by a Seliwanoff test,[16] and the yellow-brown spots produced on filter paper with the anthrone reagent.[17] Extraction with cold and hot water and with 0·5% sodium carbonate solution of the alcohol-extracted weed gave similar polysaccharide material (visual chromatographic examination of the hydrolysate).

Extraction of a Xylan.—The residual weed (from 50 g. of original dried weed), after extraction with alcohol and dilute acid, was heated to 70° in water (250 c.c.) containing glacial acetic acid (1 c.c.) and sodium chlorite [4] (4 × 3 g.) was added at hourly intervals during 4 hr. The weed was then separated at the centrifuge, washed with water and extracted with N-sodium hydroxide (125 c.c.) at room temperature with stirring during 3 days. The alkaline solution, freed from weed, was acidified with glacial acetic acid to pH 5. A gel-like precipitate was deposited which was washed with water to remove acid, followed by alcohol of increasing concentration and finally with ether. The white, powdery solid (2·5 g.), after drying over phosphoric oxide in a vacuum-desiccator, had $[\alpha]_D$ −28° (*c* 0·8 in 0·1N-NaOH) (Ash, 2·0; SO_4^{2-}, 3·6%). Hydrolysis and quantitative chromatography [14] showed that the polysaccharide contained xylose (90%) and glucose (10%).

Purification of the Xylan.—The xylan was precipitated from alkaline solution as the copper complex with Fehling's solution,[5] with copper acetate,[6] and with Benedict's solution.[1] Precipitation was also brought about with 10% aqueous cetyltrimethylammonium bromide.[7] Hydrolysates from each of the regenerated xylans contained *ca.* 10% of glucose. Suspension of the crude polysaccharide (5 g.) in water (500 c.c.) with agitation for 24 hr. gave on filtration and drying a white powder (A) (4·75 g.), $[\alpha]_D$ −31° (*c* 0·5 in 0·5N-NaOH) (Found: SO_4^{2-}, 2·4%). After dialysis against running water for 4 days the polysaccharide (4·5 g.) was isolated by freeze-drying of the aqueous suspension. This material (B) was devoid of sulphate and contained xylose 95%,[15] 97%,[14] and glucose 5%,[15] 3% [14] (ash, 0·5%). Crystalline xylose, m. p. and mixed m. p. 143—144°, $[\alpha]_D$ +41·9° (*c* 0·9), was separated from the hydrolysate in 92% yield. This was further characterised as the dibenzylidene dimethyl acetal derivative, m. p. mixed m. p. 210°. Repeated extraction of polysaccharide (B) (1·5 g.) with water finally led to the isolation of xylan (C) (1·1 g.), $[\alpha]_D$ −35°, containing only xylose residues.

Periodate Oxidation of Xylan.—(i) *Uptake of periodate.* Dry xylan (B) (361 mg.), (C) (312·4 mg.) was oxidised with 3% sodium metaperiodate (50 ml.) in the dark and the reduction of periodate was measured.[18] The moles of periodate consumed per $C_5H_8O_4$ unit were determined: (B) 0·015 (3 hr.), 0·026 (5 hr.), 0·055 (22 hr.), 0·064 (42 hr.), 0·070 (66 hr.), 0·070 (114 hr. const.). (C) 0·021 (5 hr.), 0·040 (11 hr.), 0·045 (24 hr.), 0·072 (48 hr.), 0·074 (70 hr.), 0·074 (96 hr. const.). The completely oxidised solution (170 hr.), after destruction of excess of periodate with ethylene glycol, was dialysed against distilled water until free from inorganic ions (3 days) and concentrated at 35°/15 mm. to 10—15 c.c. The oxopolysaccharide from (B) (337·8 mg., 98%), isolated from this solution by freeze-drying, was hydrolysed with N-sulphuric acid. The resulting syrup contained xylose [14] (304 mg.) and a trace of glucose.

(ii) *Determination of formic acid released.* Dry xylan (B) (498 mg.) was weighed into a dark stoppered bottle and treated with sodium metaperiodate (50 c.c.; 0·1871M) with continuous agitation. Samples (5 c.c.) were removed at intervals, treated with ethylene glycol (1 c.c.), and titrated with 0·01N-sodium hydroxide from a micro-burette (Methyl Red). The following results (moles × 10^{-2}) of $H{\cdot}CO_2H$ per $C_5H_8O_4$ unit were obtained: 1·5 hr., 0·37; 3 hr., 0·37; 22 hr., 0·75; 46 hr., 1·1; 94 hr., 1·6; 166 hr., 2·1; 262 hr., 2·3; 298 hr., 2·35; 324 hr., 2·4.

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345. *The Water-soluble Polysaccharides of* Cladophora rupestris. *Part II.*[1] *Barry Degradation and Methylation of the Degraded Polysaccharide.*

By J. J. O'Donnell and Elizabeth Percival.

Repeated successive treatment of the water-soluble polysaccharide, cladophoran, with periodate and with phenylhydrazine gave a 25% yield of a degraded polymer containing L-arabinose, D-galactose, and L-rhamnose and 15% of sulphate groups. Evidence is presented that sulphate groups are linked to sugar residues other than galactose. Methylation studies established the linkages present in the degraded material. The significance of these results in relation to the whole polymer is discussed.

The water-soluble extract from the green seaweed, *Cladophora rupestris*, is a heteropolysaccharide material, which failed to separate into simple polymers when subjected to the usual fractionation techniques. The presence of 20% of organically bound sulphate made complete methylation difficult. However, separation and characterisation of a number of the sugars in the hydrolysate of the methylated material revealed some of the linkages present in the original polysaccharide, although it was not possible to advance any general structure for the polysaccharide or to allocate the sulphate residues to any particular sugar.[1]

Repeated degradation by Barry's method [2] appeared to offer a means of obtaining information on the structure of the inner part of this molecule. A single Barry degradation involves oxidation of the polysaccharide with periodate, followed by treatment of the oxo-polysaccharide with phenylhydrazine. This treatment removes all the residues in the original molecule which contained α-glycol groups and new vicinal hydroxyl groups are exposed in the degraded polymer. The process can then be repeated, and in this way successive layers of residues are removed from the periphery of the molecule. In the present experiments the reduction of periodate and the production of formic acid were measured for each oxidation. At the same time the oxo- and degraded polymers were isolated at each stage and their constituent sugars and sulphate and nitrogen contents were determined (see Tables 1 and 2).

Table 1.

	IO_4 reduced (moles/kg.)	Formic acid (moles/kg.)	Yield g.	Yield % †	N (%)
O_1 *	2·82	1·8	18·2	89	—
O_1D_1	—	—	13·7	63	3·5
D_1O_2	1·97	0·9	9·9	80	2·7
O_2D_2	—	—	6·3	66	4·2
D_2O_3	2·52	1·0	4·8	73	2·4

* O and D refer to the respective oxo- and degraded polysaccharides isolated. The second letter indicates the nature of the last treatment and the subscripts correspond to the number of such treatments.

† In calculating percentage yields of oxo-polysaccharide, allowance is made for material consumed during measurements.

Analysis of the hydrolysate of the oxo-polysaccharide O_1 confirmed the cleavage of all the xylose and a considerable proportion of the galactose units. For these sugars to be attacked by periodate they must be present as end-group or linked only through positions 1 and 4 (and/or 6 in the case of galactose) in the original polysaccharide. Glyoxal bisphenylhydrazone was isolated from the ethereal extract of the degraded material O_1D_1. This is in keeping with the cleavage of 1 : 4-linked xylose and galactose residues. No

Measured quantities of this mixture (0·02, 0·04, 0·06, 0·08 c.c.) were applied from an Agla microburette at intervals along the starting line of a paper chromatogram (20 × 40 cm.). Also spotted on the paper were samples of the hydrolysate. After elution the chromatogram was sprayed with a 1% solution of freshly prepared *p*-anisidine hydrochloride and heated at 130° for 10 min. Each of the coloured spots which developed, together with suitable blanks, were cut out and left for 10 min. in 3 c.c. of methanolic stannous chloride (1 g. of stannous chloride dissolved in 5 c.c. of water, 90 c.c. of methanol added, and the mixture filtered). The density of the resulting solution was then measured in a Unicam spectrophotometer at the wavelength of maximum absorbance for the particular sugar being examined. When the readings for the standard solutions of the respective sugars were plotted against concentration a straight-line graph was obtained in each case, and these were used to determine the unknown materials. A complete duplication of the experiment ensured accuracy within ±5%.

The oxo-polysaccharide in the remainder of the solution was isolated by freeze-drying. The second oxidation was not complete until after 70 hr. and the third oxidation after 86 hr.

The oxo-polysaccharide was degraded by heating a 4% aqueous solution at 100° for 2 hr. with 7% acetic acid and 3% phenylhydrazine. After exhaustive extraction with ether and dialysis the degraded polysaccharide was isolated, by freeze-drying, as a light yellow powder. Further extraction with ether failed to reduce the nitrogen content.

The ethereal extracts after evaporation to dryness and extraction with glacial acetic acid afforded a brown powder. Dissolution in ether and addition of light petroleum afforded yellow crystals of glyoxal bisphenylhydrazone (0·9 g.), m. p. and mixed m. p. 167°. Removal of the acetic acid yielded crystalline *N*-acetylphenylhydrazine, m. p. and mixed m. p. 124°.

Methylation of the Degraded Cladophoran D_2O_3.—Thallium hydroxide (6 g.) and an aqueous solution of cladophoran D_2O_3 (3·6 g.; 30 c.c.) were freeze-dried and the product was refluxed overnight with methyl iodide (25 c.c.).[4] After evaporation to dryness the residue was exhaustively extracted with methanol (3 × 25 c.c.), hot 50% aqueous methanol (3 × 25 c.c.), and hot water (3 × 25 c.c.). The combined extracts were re-treated with thallium hydroxide and methyl iodide. After four methylations in all, the final residue was exhaustively extracted with chloroform. Removal of the chloroform from the combined extracts gave a brown powder (1·88 g.) (Found: OMe, 26·7%). Several methylations with Purdie reagents failed to raise the methoxyl content above 26·9%.

Hydrolysis of the Methylated Polysaccharide and Characterisation of the Methylated Sugars.—The above powder (1·80 g.) was hydrolysed under reflux with N-methanolic hydrogen chloride–water (9 : 1 by vol.) for 7 hr. Following neutralisation with silver carbonate and evaporation to dryness, the residue was thoroughly extracted with water. Removal of the water from the aqueous extracts and chloroform-extraction of the residue gave on evaporation an amorphous hydrolysate (0·96 g.). This was separated into its constituents on a cellulose column (55 × 2·3 cm.). After elution of five fractions with a water-saturated mixture of light petroleum (b. p. 60—80°)–butan-1-ol (8 : 2), the proportions were changed to 7 : 3. R_G values are recorded for paper chromatograms developed with butan-1-ol–ethanol–water (4 : 1 : 5). The products of demethylation [1] were detected by paper chromatography. The following fractions were collected:

Fraction 1, a yellow syrup (213 mg.) of R_G 1·05 (Found: N, 14·3%).

Fraction 2, syrupy 2 : 4-di-*O*-methylrhamnose (40 mg.), R_G 0·83, $[\alpha]_D$ −17° (*c* 0·3) (Found: OMe, 33·6. Calc. for $C_8H_{16}O_5$: OMe, 32·3%); demethylation gave only rhamnose; nucleation with an authentic specimen afforded needles of 2 : 4-di-*O*-methylrhamnose [9] with m. p. and mixed m. p. 82—83°.

Fraction 3, syrupy 2 : 4-di-*O*-methylarabinose (162 mg.), R_G 0·67, $[\alpha]_D$ +28° (*c* 2·0) (Found: OMe, 34·9. Calc. for $C_7H_{14}O_5$: OMe, 34·8%); demethylation of a portion of the syrup gave only arabinose; the derived anilide [10] had m. p. 126° and gave an *X*-ray powder photograph identical with that of 2 : 4-di-*O*-methyl-*N*-phenyl-L-arabinosylamine.

Fraction 4, syrupy 2 : 4 : 6-tri-*O*-methylgalactose (143 mg.), R_G 0·64, which crystallised from methanol; the crystals had m. p. 116°, $[\alpha]_D$ +88° (*c* 0·92) [11] (Found: OMe, 41·7. Calc. for $C_9H_{19}O_6$: OMe, 41·9%); demethylation gave only galactose.

Fraction 5, syrupy 2-*O*-methylarabinose (37 mg.), R_G 0·43, $[\alpha]_D$ +74° (Found: OMe, 18·0. Calc. for $C_6H_{12}O_5$: OMe, 17·8%); this was chromatographically and ionophoretically identical with authentic 2-*O*-methylarabinose and in keeping with this structure failed to give a red spot on spraying of a paper chromatogram with triphenyltetrazolium hydroxide; [12] the derived phenylhydrazone had m. p. 115°.

Fraction 6, crystalline L-rhamnose (89 mg.), R_G 0·30, $[\alpha]_D$ +8·2 (const.), m. p. and mixed m. p. 68°.

Fraction 7, syrupy 6-*O*-methylgalactose (23 mg.), R_G 0·23, $[\alpha]_D$ +74° (*c* 1·1) (Found: OMe, 15·1. Calc. for $C_7H_{14}O_6$: OMe, 16·0%); demethylation gave only galactose; the derived phenylhydrazone had m. p. 117°.[13]

Fraction 8, crystalline D-galactose (46 mg.), R_G 0·10, $[\alpha]_D$ +80° (const.), m. p. and mixed m. p. 162—164°.

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433. *Structural Investigations on the Water-soluble Polysaccharides from the Green Seaweed* Acrosiphonia centralis (Spongomorpha arcta).

By J. J. O'Donnell and Elizabeth Percival.

Water-soluble sulphated polysaccharides containing D-glucose, D-xylose, L-rhamnose, and D-glucuronic acid, together with small amounts of D-galactose and D-mannose, have been extracted from *Acrosiphonia centralis*. Chloroform-extraction of the acetylated material separates *ca.* 9% of a glucose-rich fraction resembling starch. Further study of the main polysaccharide material revealed the presence of end-group xylose, 1 : 4-linked xylose and rhamnose. and a relatively large proportion of triply linked rhamnose. Sulphate groups are attached to rhamnose and/or xylose residues. All the uronic acid residues appear to be present as end-groups linked to rhamnose. 4-*O*-β-D-Glucopyruronosyl-L-rhamnose was separated and characterised. This polysaccharide is compared with the water-soluble polysaccharides of *Cladophora rupestris* and of *Ulva lactuca*.

In view of the close botanical relation between the green seaweeds, *Cladophora rupestris* and *Acrosiphonia centralis* (*Spongomorpha arcta*), and of the studies on the water-soluble polysaccharide of the former,[1] it seemed desirable to determine whether *A. centralis* synthesised similar material. Collection of pure species of the latter weed is difficult, and we are grateful to Dr. R. Lewin who sent us from Nova Scotia sufficient material for a preliminary investigation. It was at once apparent that the two water-soluble extracts were different and that large-scale investigations of *A. centralis* were warranted. Mr. H. Powell very kindly collected pure species of this weed from a single area at Millport, and the structural investigations now reported have been carried out on the water-soluble polysaccharides isolated from this material.

After removal of free sugars and most of the colouring matter by extraction with aqueous ethanol, water-soluble polysaccharide material was separated (mainly as the ammonium salt) from the residual weed by extraction with aqueous ammonium oxalate. Relatively little glucose remained in the weed after this extraction and it follows therefore that the cellulose content of *A. centralis* must be very small.

In Table 1 the properties of the water-soluble extract and the molar proportions of the sugars present in a partial hydrolysate are compared with those for similar extracts from

Table 1.

Molar proportions	*Cladophora rupestris* [1]	*Acrosiphonia centralis*	*Ulva lactuca* [2]
Arabinose	3·7	—	—
Galactose	2·8	0·1	—
Xylose	1·0	1·6	1·3
Rhamnose	0·4	1·4	4·4
Glucose	0·2	1·0	1·0
Mannose	—	0·2	—
Uronic acid (%)	3·0	19·3	20·8
SO_4^{2-} (%)	16·1	7·8	17·5
Ash (%)	13·7	10·0	19·0
$[\alpha]_D$ (in H_2O)	+69°	−31°	−47°

Cladophora rupestris [1] and from *Ulva lactuca*.[2] The neutral sugars in the partial hydrolysate from *A. centralis* were separated and characterised as crystalline sugars or their derivatives. Hydrolysis for longer periods or with more concentrated acid led to extensive degradation. The sulphate content (3·8%) calculated from the sulphated ash comprises half the total sulphate (7·8%), providing evidence of sulphate esters in the polysaccharide.

The water-soluble polysaccharide material from *C. rupestris* (see Table 1) has a positive

rotation and consists mainly of arabinose, galactose, and xylose units: the polysaccharides from the two other green seaweeds have negative rotations and comprise mainly glucose, xylose, rhamnose, and uronic acid residues. Although all three extracts contain ester sulphate groups it is plain that the extract from *A. centralis* more nearly resembles that from *U. lactuca*. The difference in the relative proportions of the sugars in the two extracts containing much uronic acid may be due, at least in part, to the resistance of polyuronides to hydrolysis; the *A. centralis* hydrolysate comprises *ca.* 45% of oligouronic acids containing uronic acid and sugar residues.

The sulphate (7·8%) and uronic anhydride (20%) in *A. centralis* extract correspond to about one residue to every eight and four sugar units respectively. A hydrolysate with 20% of uronic acid, present as unhydrolysed barium uronosylaldose, would contain *ca.* 16% of aldose and 6% of barium; *i.e.*, 40—45% of the neutralised hydrolysate would consist of barium uronates, which is in agreement with the experimental findings.

The free-acid polysaccharide has an equivalent weight of 459. In the absence of sulphate and ash, a 20% uronic acid content corresponds to an equivalent weight of 870. A sulphate content of 7·8% represents 67 g. (0·70 equivalent) of sulphate in 870 g. Hence 870 g. correspond, not to 1, but to 1·70 equivalents, and the calculated equivalent weight of a polysaccharide containing 20% of uronic acid and 7·8% of sulphate is 870/1·70, *i.e.*, 512. The uronic acid and sulphate content were determined on neutral polysaccharide which contained 10% of ash; it is permissible therefore to deduct 10% in the above calculation of the theoretical equivalent weight. The final calculated value then becomes 461, in excellent agreement with the experimental value.

The periodate consumption by the polysaccharide corresponds to *ca.* 1 mole, and the formic acid release to *ca.* 0·6 mole, for every anhydro-sugar residue. These results indicate that many of the units have free contiguous hydroxyl groups. An oxopolysaccharide was isolated, after dialysis, in 68% yield, and hydrolysis showed that this contained intact xylose and rhamnose units. There is, therefore, an indication of the presence of either 1 : 3-linked units or of branch points in the molecule.

Attempted fractionation of the free polysaccharide was unsuccessful. Acetylation, followed by chloroform-extraction, gave, however, a glucose-rich acetate (A) (9%), $[\alpha]_D$ +71° (devoid of sulphate). The deacetylated material resembled starch in its reactions with iodine. Methylation of this acetate (A) and hydrolysis of the product, followed by separation and characterisation of the methylated sugars, confirmed this structure, and also indicated some branching at position 6 of the sugar units. 2 : 3 : 4 : 6-Tetra- (1 part), 2 : 3 : 6-tri- (4 parts), and 2 : 3-di-*O*-methylglucose (1 part) were separated and identified. 2 : 3 : 6-Tri-*O*-methylmannose (*ca.* 10%) was also separated from this hydrolysate, proving the presence of 1 : 4-linked mannose residues, and suggesting the presence of a small quantity of a mannan or glucomannan. The presence of small amounts of 2 : 3 : 4-tri- and 2 : 3-di-*O*-methylxylose and of methylated acids is considered to be due to incomplete separation of the main polysaccharide material.

After removal of the glucose-rich fraction from the acetylated *A. centralis* extract the residual material (B), $[\alpha]_D$ −38°, was methylated and the product (OMe, 35·6%) hydrolysed. 2 : 3 : 4-Tri-*O*-methylxylose (1 part), 2 : 3-di-*O*-methylxylose (3 parts), 2 : 3-di-*O*-methylrhamnose (3 parts), and 2-*O*-methylrhamnose (5 parts), together with small quantities of 2 : 3 : 6-tri- and 2 : 3-di-*O*-methylglucose, were separated and characterised. Since both the methylated glucoses separated from this fraction were also present in the hydrolysate of the methylated fraction (A), their presence in both fractions is probably due to incomplete separation of the glucan from the polysaccharide(s) acetate.

These observations reveal structural similarities between the polysaccharides of the two weeds, *Ulva* and *Acrosiphonia*. Both polysaccharides carry xylose end groups and have 1 : 4-linked xylose and rhamnose. The isolation of a monomethylrhamnose, in relatively large quantity, from the methylated *Acrosiphonia* extract, is evidence that rhamnose occurs at branch points in the molecule.

In spite of the use of a wide variety of hydrolytic conditions no-one, so far as the authors are aware, has yet succeeded in isolating glucuronic acid or its lactone from any of the green seaweeds, the conditions required for complete hydrolysis invariably leading to degradation. Fractionation of the acidic material from the partial hydrolysate of the extract from *A. centralis* led to the separation of di- (10%), tri- (4%), and tetra-oligouronic acids (3%). A large polymeric fraction (65%) was also isolated, prolonged acid hydrolysis of which afforded the original mixture of oligouronic acids. Glycosidation, reduction, and hydrolysis of the first three fractions gave syrups consisting of varying proportions of glucose and rhamnose. The syrup from the first fraction had $[\alpha]_D$ $-6°$ and was shown by quantitative chromatography [3] to contain equimolar proportions of D-glucose and L-rhamnose, the former being characterised by oxidation by the specific enzyme,[4] glucose oxidase, and the latter by formation of the authentic crystalline benzoylhydrazone. It is considered therefore that the glucose arose from the reduction of glucuronic acid residues. The ester glycoside of this fraction consumed 2·8 moles of periodate per mole, indicating that linkage occurred through position 2 or 4 of the rhamnose residue; methyl 2- or 4-glucuronosylrhamnoside requires 3 mols. of periodate for complete oxidation, whereas methyl 3-glucuronosylrhamnoside reduces only 2 mols. Methylation of the reduced ester glucoside confirmed these findings. Crystalline 2 : 3 : 4 : 6-tetra-*O*-methylglucose and syrupy 2 : 3-di-*O*-methylrhamnose were separated from the hydrolysate of the methylated disaccharide. The tetra-*O*-methylglucose could only have arisen from glycosidically linked glucuronic acid, and the biuronic acid has therefore the constitution 4-*O*-β-D-glucopyruronosyl-L-rhamnose, the β-configuration being inferred from the negative specific rotation. The chromatographic mobility ($R_{glucose}$ *ca.* 1·0) of this fraction is high for an aldobiuronic acid (cf. 2-*O*-D-glucuronosyl-L-rhamnose, $R_{glucose}$ 0·24) and it seemed likely that, in the free state, it existed as the lactone. Chromatographic support for this conclusion was obtained, inasmuch as it behaved in the same way as glucurone on similar treatment. Both migrated back from the starting line on ionophoresis at pH 5·5, indicating the absence of charged groups at this pH, whereas at pH 10 their respective M_G values are 0·75 and 0·96; in addition they each gave two spots if treated with ammonia before development. In this connection it is worth noting that the equivalent weight (328) of this fraction is closer to the calculated value (322) for the lactone of glucuronosylrhamnose than to the calculated value for the free biuronic acid (340).

The trisaccharide had equivalent weight 249 and molecular weight 508: a diglucuronosyl-*O*-rhamnose would require 257 and 514 respectively. In agreement with this structure, glycosidation, reduction, and hydrolysis afforded glucose and rhamnose in the molar proportions of *ca.* 2 : 1.

The tetrasaccharide fraction was difficult to purify and, although equimolar proportions of glucose and rhamnose were obtained on hydrolysis of the reduced material, the values for the equivalent and molecular weights cannot be explained on this basis and further evidence must be awaited.

Reduction and hydrolysis of the glycoside ester of the acidic polymeric fraction afforded equimolar proportions of glucose and rhamnose, together with a very small amount of xylose. The low yield (24%) from this reaction is attributable to the low solubility of the material in the organic solvents employed for reduction.

Methylation studies of the acidic material confirmed these results. After removal of the neutral methylated sugars the methylated oligouronic acids were separated on thick paper. After glycosidation, reduction, and hydrolysis, the first fraction gave equimolar proportions of 2 : 3 : 4-tri-*O*-methyl-D-glucose and 2-*O*-methyl-L-rhamnose. This fraction therefore consisted of 2 : 3 : 4-tri-*O*-methyl-4-*O*-D-glucopyruronosyl-2-*O*-methyl-L-rhamnose and almost certainly originated from the same portion of the molecule as the 4-*O*-D-glucopyruronosyl-L-rhamnose, isolated from the hydrolysate of the unmethylated extract. Furthermore it follows that these units must be linked to the rest of the molecule through the hydroxyl group on position 3 of the rhamnose. Each of the remaining acidic fractions

was similarly treated and each gave rise to approximately equimolar proportions of 2 : 3 : 4-tri-*O*-methylglucose and a non-reducing syrup with a high chromatographic mobility (R_G 1·15). The latter material did not reduce periodate and gave a positive test for methoxyl. These properties are in agreement with the structure, 1 : 4-anhydro-2-*O*-methylrhamnitol. Although it has been shown that rhamnitol, on treatment with acid, gives the 1 : 4-anhydro-derivative [5] it is difficult to see how this product could have arisen from the methylated acid fraction. Only if cleavage of the glycosidic links occurred before or during the reduction would rhamnitol be produced. Although comparison with reduced 2-*O*-methylrhamnose suggests that this non-reducing syrup derived from the acid fraction probably arose from rhamnose residues in the polysaccharide its characterisation as 1 : 4-anhydro-2-*O*-methylrhamnitol can only be regarded as tentative.

Methylation failed to reduce the proportion of sulphate groups in the polysaccharide (B). The allocation of these groups to individual sugar residues is a difficult problem; their attachment to glucose residues is very unlikely in view of the separation of a sulphate-free glucan and of the small amount of glucose in polysaccharide (B). The reduced glucuronic acid residues were isolated solely as 2 : 3 : 4-tri-*O*-methylglucose and must therefore occur as glycosidically linked end-groups with all the hydroxyl groups free for methylation. This leaves only xylose and/or rhamnose for union with the sulphate groups. 2 : 3-Di-*O*-methylxylose and 2 : 3-di-*O*-methylrhamnose were isolated in approximately equal quantities together with about twice as much mono-*O*-methylrhamnose. There are barely enough xylose residues to accommodate all the sulphate groups. Furthermore, if sulphate is linked to position 4 of those xylose units isolated as 2 : 3-di-*O*-methylxylose then practically all the xylose residues in the molecule are present as end-groups, many of which are sulphated. A likely site, therefore, for some at least of the sulphate groups, is on the rhamnose residues.

The polysaccharide(s) (B) must have a highly complicated structure. At least part of the molecule consists of 1 : 3-linked rhamnose residues carrying glucuronic acid units linked at position 4. The results of methylation studies have revealed the presence of the following residues:

GA...	...4 R 1...	...4 R 1... 3 ⋮	...4 X 1...	X 1...	...4 G 1...	...4 G 1... 6 ⋮
(25%)	(11%)	(21%)	(11%)	(4·2%)	(1·7%)	(0·6%)

GA = glucuronic acid, G = glucose, R = rhamnose, X = xylose.

The rest of the material (*ca.* 24%) comprises the non-reducing material isolated from the acid fraction.

Experimental

Analytical Methods.—All solutions were evaporated under reduced pressure below 60°. Paper partition chromatography was carried out on Whatman No. 1 paper with upper layers of the following solvent systems (v/v) (1) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (2) ethyl acetate–pyridine–water (10 : 4 : 3); (3) ethyl acete–acetic acid–formic acid–water (18 : 4 : 1 : 5); (4) ethyl acetate–acetic acid–water (3 : 1 : 3); (5) butan-1-ol–acetic acid–water (4 : 1 : 5); (6) butan-1-ol–ethanol–water (4 : 1 : 5); (7) ethyl methyl ketone half saturated with water plus ammonia (99 : 1); (8) ethyl acetate–acetic acid–water (9 : 2 : 2). Papers were sprayed with a saturated aqueous solution of aniline oxalate. R_G, R_{Gl}, G_{GA}, and R_F are the rates of travel relative to tetramethylglucose, glucose, glucuronic acid (glucurone co-spotted with ammonia), and the solvent front respectively. Ionophoresis [6] was carried out (750 v; 12·5 ma) in borate buffer at pH 10, and the ionophoretograms were sprayed with saturated aqueous aniline oxalate containing 20% of acetic acid. Specific rotations were measured for solutions in water at 18°.

Acrosiphonia centralis (100 g. dry), collected from Millport in April and June 1957, was thoroughly washed with sea-water, dried in a current of air at room temperature, and extracted under reflux with 85% aqueous ethanol until the alcoholic extracts were colourless. The

residual weed was then exhaustively extracted at 95° with 1% ammonium oxalate solution and with water, and at room temperature with 0·01N-hydrochloric acid, 2% aqueous sodium carbonate, and 5% aqueous potassium hydroxide. The extracts were each dialysed until free from inorganic material, and the polysaccharides isolated by freeze-drying. Chromatography [3] of the hydrolysate of each extract revealed that they all contained the same sugar residues, and in approximately the same proportions. The weed remaining after extraction with ammonium oxalate was kept for 10 days in 72% sulphuric acid solution.[7] The filtered solution was diluted with water to a strength of N, then heated at 100° for 7 hr. The resulting syrup contained glucose : xylose : rhamnose (1 : 1 : 1) together with minute quantities of oligouronic acids (paper chromatography, visual examination).

Extraction with ammoniun oxalate gave the highest yield (12% of the dry weight of weed) of water-soluble polysaccharide, and a comparison of the properties of this extract with those of the hot water extract (4·5% yield) demonstrated the essential similarity of the two materials:

Extract	Ash (%)	Sulphate (%)	Nitrogen (%)
Hot water	13·9	7·8	1·0
Ammonium oxalate	10·0	7·8	4·2

In view of these findings all the subsequent investigations were carried out on an ammonium oxalate extract.

The polysaccharide (12 g.) was isolated, as an off-white powder, by freeze-drying. It had $[\alpha]_D$ −31° (*c* 1·2) [Found: N (as ammonium salt by direct distillation with alkali) 3·6, (Kjeldahl) 4·2; ash (direct) 10·0; (as sulphate) 11·8; SO_4^{2-} in ash 32; total SO_4^{2-}, 7·8; uronic anhydride,[8] 20·3%].

Attempted Fractionation of the Aqueous Extract.—10% Aqueous cetyltrimethylammonium bromide (5 c.c.) was added dropwise, with stirring, to an aqueous solution of the extract (189 mg. in 20 c.c.). Regeneration [9] of the polysaccharide from the complex and from the supernatant liquid gave 132 mg. (SO_4^{2-}, 8·1%) and 37 mg. (SO_4^{2-}, 9·5%) respectively. Four successive fractionations in all were carried out; in each case hydrolysates of material both from the complexes and the supernatant layers revealed the presence of glucose, xylose, and rhamnose with no significant difference in their relative proportions (paper chromatography).

Treatment of the extract with aqueous solutions of copper sulphate and copper acetate failed to achieve fractionation.[10]

Equivalent-weight Determination.—An aqueous solution of the polysaccharide (133 mg. in 20 c.c.) was recycled four times through a column of Amberlite IR-120H resin (10 g.), and the eluant freeze-dried to a white powder (Found: ash, nil; SO_4^{2-}, 7·5%). The equivalent weight of this material, determined by titration with 0·01N-sodium hydroxide, was 459 (mean of several determinations).

Hydrolysis of the Polysaccharide.—(*a*) The polysaccharide (0·335 g.) was hydrolysed with N-sulphuric acid (30 c.c.) at 100°. The course of the hydrolysis was followed by chromatography and measurement of specific rotation and reducing power (hypoiodite reduction [10]) on aliquot parts:

Time (hr.)	0	0·5	1	2	4	6	8
Iodine (0·01N; c.c.)	1·9	5·4	7·1	8·6	9·7	—	—
$[\alpha]_D$	−29°	−12°	−4°	+2°	+6°	+4°	+6°

Chromatography (solvent 1) revealed that glucose, xylose, and rhamnose were released within the first hour, accompanied by traces of neutral oligosaccharides; the oligosaccharides had entirely disappeared in 8 hr. The presence of uronic acids was indicated by a series of pink spots near the starting line. Traces of mannose and galactose were also visible.

(*b*) The polysaccharide (1 g.) was heated under reflux with 3% methanolic hydrogen chloride (25 c.c.) for 6 hr.; 15% methanolic hydrogen chloride (15 c.c.) was then added at 16-hourly intervals, four times in all (total hydrolysis time 70 hr.). Water (30 c.c.) was added and the mixture heated at 100° for 6 hr. Chromatography in solvent (3) failed to reveal the presence of glucuronic acid or its lactone.

(*c*) The polysaccharide (1 g.) was heated with 98% formic acid (50 c.c.) under reflux in nitrogen for 7 hr. The neutral sugars were separated by elution through a column of IR-45B resin (acetate form), and the column was washed with water until the eluant gave a negative Molisch reaction. The acid fraction was recovered by elution with N-formic acid. After

repeated extraction with ether the aqueous-acid fraction was evaporated to dryness and exhaustively extracted with ethanol. Chromatography (solvent 3) of the concentrated ethanolic extracts revealed spots of R_{Gl} 1·05, M_G 0·75, and R_{Gl} 0·88 and 0·63 respectively (cf. glucurone R_{Gl} 2·4).

Partial Hydrolysis of the Polysaccharide and Separation of the Sugars.—The polysaccharide (3·25 g.) was hydrolysed with N-sulphuric acid for 7 hr. at 100°. After neutralisation of the cooled solution with barium carbonate, the hydrolysate (2·2 g.) was obtained as an amorphous solid. Quantitative analysis of the neutral sugars in this hydrolysate by a colorimetric method [3] gave the molar proportions of glucose (1·0), xylose (2·1), rhamnose (1·3); and by sodium periodate oxidation [11] glucose (1·0), galactose (0·12), mannose (0·21), xylose (2·2), and rhamnose (1·6). A portion of the hydrolysate (1·75 g.) was fractionated on a cellulose column (2·8 × 60 cm.) with butan-1-ol half saturated with water as eluant. R_F values are recorded for solvent (1).

Fraction 1. A yellow syrup (28·8 mg.), R_F 0·09, $[\alpha]_D$ −4° (*c* 2·29) (Found: OMe, nil), gave a positive Selivanoff test.

Fraction 2. Crystalline L-rhamnose hydrate (139 mg.), m. p. and mixed m. p. 68°, R_F 0·55; the derived benzoylhydrazone had m. p. and mixed m. p. 186°.

Fraction 3. A syrup (18·5 mg.), R_F 0·46, was chromatographically distinct from ribose and gave a positive Selivanoff test.

Fraction 4. Crystalline D-xylose (264 mg.), m. p. and mixed m. p. 145°, R_F 0·44, $[\alpha]_D$ +19° (*c* 1·0). The derived dibenzylidene dimethyl acetal had m. p. 186°.

Fraction 5. A syrup (39·0 mg.), R_F 0·38. Although contaminated with xylose it was mainly mannose (solvents 1 and 3); its identity was confirmed by isolation of mannose phenylhydrazone m. p. and mixed m. p. 188°.

Fraction 6. A syrup (56·6 mg.), R_F 0·33, $[\alpha]_D$ +53° (*c* 0·25); its identity as D-glucose was established by formation of the dichlorophenylhydrazone, m. p. and mixed m. p. 153°.

Fraction 7. A syrup (21 mg.), R_F 0·28, chromatographically identical with galactose (solvents 1 and 3). The derived diethyl mercaptal had m. p. and mixed m. p. with the diethyl mercaptal of D-galactose, 140—142°.

Fraction 8. Eluted with water, and isolated as an amorphous solid (820 mg.), R_F 0—0·07.

Examination of the acidic fraction. Fraction 8 (2·14 g., the combined products from two separations) was converted into the free acid (1·57 g.) by treatment with IR-120(H^+) resin and an aqueous solution (10 c.c.), mixed to a slurry with cellulose powder, and freeze-dried. The product was applied to the top of a cellulose column (74 × 3·6 cm.) and eluted with solvent (8); R_{Gl} values recorded below are for solvent (3). Seven fractions were separated. Each of the fractions, after evaporation to small volume (10 c.c.), was treated with barium hydroxide (to remove traces of sulphuric acid) and filtered. The filtrate was exhaustively treated with ether in a continuous extractor to remove final traces of acetic acid. Deionisation with IR-120(H^+) resin afforded clear acidic syrups.

(*a*) Fraction 1 (0—2·5 l.) (144 mg.). Chromatography showed glucose, xylose, and rhamnose together with considerable streaking.

(*b*) Fraction 2 (2·5—3·6 l.). An aldobiuronic acid (227 mg.), $[\alpha]_D$ −6° (*c* 0·5), R_{Gl} 1·05, R_{GA} 0·85 (solvent 4); co-spotting with ammonium hydroxide before elution gave a second spot, R_{Gl} 0·57 (cf. D-glucurone R_{Gl} 2·4 and 1·0 under these conditions), M_G (in borate buffer pH 10) 0·75, (in acetate buffer pH 5·5) 0·0 (cf. glucose and glucurone which behaved similarly). Co-spotting with ammonia before ionophoresis in acetate buffer gave a second spot *ca.* 15 cm. from the starting line. Galacturonic acid in acetate buffer moves *ca.* 14 cm. in 5 hr. (Found: equiv., 328, by titration). The reducing power, compared with that of rhamnose monohydrate as standard, was measured by treatment with iodine in excess of sodium hydroxide solution. The mixture was set aside for 2 hr., then acidified with sulphuric acid and titrated with sodium thiosulphate. The aldobiouronic acid (12·4 mg.) had the reducing power of 6·55 mg. of rhamnose monohydrate. This corresponds to a molecular weight of 344. Hydrolysis of a portion with 2N-sulphuric acid gave only degradation products of the furfuraldehyde type (paper chromatography). A portion (204 mg. and 50 mg. from a previous fractionation) was heated under reflux with 2% methanolic hydrogen chloride for 6 hr. The resulting ester glycoside (266 mg.) was suspended in tetrahydrofuran (30 c.c.) which had been dried by distillation from sodium and from lithium aluminium hydride. A saturated suspension of the hydride in tetrahydrofuran (6 c.c.) was then added dropwise, and the solution refluxed for 1 hr.

Dropwise addition of water (18 c.c.) was followed by filtration of precipitated aluminium hydroxide and treatment with IR-120(H^+) resin. After evaporation of the organic liquid the remaining aqueous solution (R) was diluted with distilled water to 50 c.c. To a portion of this (10 c.c.) an equal volume of 2N-sulphuric acid was added, and the mixture heated at 100° for 6 hr. Deionisation with IR-4B(OH^-) resin, evaporation to dryness, and methanol-extraction of the residue afforded a syrup (33·7 mg.). Chromatography (solvents 1 and 3), together with colorimetric estimation, revealed the presence of glucose and rhamnose in the molar proportion of 1 : 0·95. The hydrolysed material (30 mg.) was treated with glucose oxidase,[4] and the enzyme was later removed by addition of equivalent solutions of cadmium sulphate and barium hydroxide and filtration of the resulting precipitate. Evaporation followed by methanol-extraction yielded a syrup which contained only rhamnose (paper chromatography). The derived benzoylhydrazone had m. p. and mixed m. p. 185°.

A portion of the diluted solution (R) (4 c.c.) was deionised with IR-120(H^+) and IR-4B(OH^-) resin and evaporated to a clear glass (14·2 mg.) which was oxidised with 0·015M-sodium periodate (10 c.c.). The reduction of periodate after 44 hr. was found [12] to be equivalent to 2·8 moles of periodate per mole of disaccharide glycoside and proceeded only very slowly beyond this figure.

The remainder of solution (R) (containing 128 mg.) was methylated with 25% aqueous sodium hydroxide in nitrogen at 5°, and dimethyl sulphate (17 c.c.) was added dropwise with cooling and stirring during 6 hr. Next morning the mixture was remethylated twice more and then extracted with chloroform. Evaporation of the chloroform extracts afforded the reduced methylated disaccharide glycoside (98 mg.), $[\alpha]_D$ +7° (*c* 2·0 in methanol). Hydrolysis with aqueous-methanolic N-hydrogen chloride (1 : 1) gave a syrup (84 mg.) which was separated on 3MM paper (40 × 40 cm.) by elution with solvent (7). R_G values are recorded below for solvent (6).

Fraction (i). 2 : 3 : 4 : 6-Tetra-*O*-methylglucose (36 mg.), m. p. and mixed m. p. 94°, $[\alpha]_D$ 79° (*c* 0·9), R_G 1·0 (Found: OMe, 51·9. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%).

Fraction (ii). 2 : 3-Di-*O*-methylrhamnose (34 mg.), $[\alpha]_D$ +42° (*c* 2·9), R_G 0·86, M_G 0·02. Oxidation with periodate and chromatography of the product [13] revealed one spot, of R_F 0·81. Comparison with the oxidation product of authentic 2 : 4-di-*O*-methylrhamnose revealed spots of R_F 0·72, 0·78, and 0·85 (solvent 6). Cf. page , fraction 2.

(*c*) Fraction 3 (3·6—4·4 l.) (58 mg.). Chromatography showed this to be a mixture of fractions 2 and 4.

(*d*) Fraction 4 (4·4—5·2 l.) (45 mg.), $[\alpha]_D$ +4° (*c* 0·5), R_{Gl} 0·88, R_{GA} 0·68 (solvent 8) (Found: equiv. 514, by titration; *M*, 508. Calc. for diglucuronosyl-*O*-rhamnose $C_{18}H_{28}O_{17}$: *M*, 516). Conversion into the ester glycoside and reduction, as for fraction 2, gave on hydrolysis a syrup consisting of glucose : rhamnose [3] 1·76 : 1.

(*e*) Fraction 5 (5·2—5·6 l.) (27 mg.) was a mixture of fractions 4 and 6.

(*f*) Fraction 6 (5·6—6·4 l.). The syrup (51 mg.), R_{Gl} 0·63, R_{GA} 0·44 (solvent 8) (Found: equiv. 690, by titration; *M*, 1050), on glycosidation, reduction, and hydrolysis, yielded a syrup containing glucose : rhamnose = 1 : 0·83.

(*g*) Fraction 7 (aqueous eluant) (974 mg.), R_{Gl} 0—0·4. Further hydrolysis (16 hr., 2N-HCl at 100°) of a portion of this syrup and chromatography of the hydrolysate revealed spots corresponding to the acidic oligosaccharides described above. Esterification, reduction, and hydrolysis afforded a syrup containing glucose : rhamnose = 1 : 0·88, together with a trace of xylose.

Periodate Oxidation of the Extract.—The extract (0·153 g.) in water (30 c.c.) was treated with 0·095M-sodium periodate (30 c.c.). The reduction of periodate and release of formic acid were measured on samples (1 c.c.) withdrawn at intervals: [1]

Time (hr.)	1·25	3·5	8	25	32	48	96
Moles of periodate reduced/anhydro-unit	0·47	0·61	0·70	0·90	0·98	1·08	1·20
Moles of formic acid released/ ,,	0·138	0·177	0·215	0·250	0·256	0·397	0·586

Oxidation was stopped after 96 hr. by passing sulphur dioxide through the cooled mixture. After dialysis, freeze-drying afforded the oxopolysaccharide (204 mg.) (Found: ash, 6·7; SO_4^{2-}, 8·6%). Chromatography of the hydrolysate (7 hr., N-H_2SO_4 at 100°)revea led the presence of small amounts of xylose and rhamnose.

Acetylation of the Polysaccharide.—The polysaccharide (13·8 g.), dispersed in formamide (100 c.c.) and pyridine (700 c.c.), was shaken with acetic anhydride (200 c.c.) during 72 hr. The mixture was added slowly with cooling to an equal volume of water. After dialysis and evapor-

ation to small volume the polysaccharide (15·6 g.) was isolated by freeze-drying (Found: Ac 13·6%). Reacetylation gave an off-white powder (14·5 g.) (Found: Ac, 22·4%). Further treatment with acetic anhydride failed to increase the acetyl content. The acetylated material (12·7 g.) was exhaustively extracted with chloroform. Addition of light petroleum to the concentrated chloroform extracts afforded a white precipitate (polysaccharide acetate A; 0·91 g.). The chloroform-insoluble material (11·5 g.) will henceforth be called polysaccharide acetate (B).

Polysaccharide (A).—Polysaccharide acetate (A) had $[\alpha]_D$ +71° (*c* 1·2 in $CHCl_3$) (Found: SO_4^{2-}, nil). Deacetylation of a portion with sodium methoxide afforded a polysaccharide which gave a purple colour with iodine. This property was destroyed by prior treatment with α-amylase.[14] Hydrolysis of a portion and determination [3] of the molar proportions of the sugars present gave glucose : mannose : xylose = 1·0 : 0·31 : 0·17.

Deacetylation and methylation. The acetate (A) (0·82 g.), dissolved in ice-cooled 30% aqueous sodium hydroxide (20 c.c.), was treated dropwise with dimethyl sulphate (9 c.c.) with stirring in nitrogen during 6 hr. After being kept overnight the mixture was treated with a further quantity of sodium hydroxide and dimethyl sulphate as before.[15] The mixture was neutralised (pH 7) with sulphuric acid and dialysed until free from inorganic ions. Thallium hydroxide (2 g.) was added and the mixture freeze-dried. The resulting white powder was refluxed with methyl iodide (10 c.c.) until the solution was no longer alkaline (16 hr.).[16] After evaporation of the methyl iodide the residue was extracted at room temperature with methanol (3 × 25 c.c.), and with 50% aqueous methanol (3 × 25 c.c.); under reflux with 50% aqueous methanol (3 × 25 c.c.) and with water (3 × 25 c.c.). The combined extracts were methylated as before. After a third methylation and removal of the methyl iodide the residue was extracted with chloroform. Removal of the chloroform afforded a syrup (0·34 g.). Methylation with Purdie reagents twice gave a methylated product (0·324 g.) with $[\alpha]_D$ +42° (*c* 1·03 in $CHCl_3$) (Found: OMe, 38·6%). Hydrolysis with 4% methanolic hydrogen chloride (20 c.c.) under reflux for 3·5 hr. was followed by addition of N-hydrochloric acid (20 c.c.) and heating during a further 3·5 hr. The resulting syrup (0·28 g.) was fractionated on a cellulose column (1·9 × 45 cm.) with water-saturated light petroleum–butan-1-ol (7 : 3). R_G values are recorded below for solvent (6). The products of demethylation were identified by paper chromatography.[1]

Fraction 1 (1—71 c.c.). Syrupy 2 : 3 : 4 : 6-tetra-*O*-methylglucose (21 mg.), R_G 1·0, $[\alpha]_D$ +73° (*c* 0·24) (Found: OMe, 51·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). Demethylation gave only glucose. The derived aniline derivative had m. p. and mixed m. p. 135°.

Fraction 2 (71—189 c.c.). A syrup (11·2 mg.) which revealed spots of R_G 1·0, 0·91, and 0·81, corresponding to 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methylglucose and 2 : 3 : 4-tri-*O*-methylxylose respectively, on chromatography. Demethylation gave glucose and xylose.

Fraction 3 (190—364 c.c.). A syrup (84 mg.), R_G 0·81, chromatographically identical with 2 : 3 : 6-tri-*O*-methylglucose in solvents (6) and (7), and having $[\alpha]_D$ +72° (*c* 0·78) (Found: OMe, 40·3. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). It gave only glucose on demethylation. An aliquot part (*c* 1·12) was kept at room temperature in 0·1N-methanolic hydrogen chloride and the change in rotation observed:

Time (hr.)	0·2	1	4	5	21	29	40	45	
$[\alpha]_D$.....	+0·74°	+0·52°	+0·26°	+0·24°	−0·19°	−0·25°	−0·32°	−0·32°	Final $[\alpha]_D$ −0·29°

Hydrolysis with 0·5N-hydrochloric acid gave the parent sugar. The derived lactone had $[\alpha]_D$ +33°.

Fraction 4 (379—469 c.c.). A syrup (16·5 mg.), R_G 0·76 (cf. 2 : 3-di-*O*-methylxylose, R_G 0·76; 2 : 4-di-*O*-methylxylose R_G 0·69), M_G 0·02, $[\alpha]_D$ +9° (*c* 0·7); it gave only xylose on demethylation.

Fraction 5 (470—553 c.c.) (19 mg.), R_G 0·76. Demethylation gave mannose and xylose.

Fraction 6 (554—1036 c.c.). A syrup (16 mg.), $[\alpha]_D$ +16° (*c* 0·8 in MeOH), R_G 0·76 identical with 2 : 3 : 6-, and distinct from 2 : 4 : 6- and 3 : 4 : 6-tri-*O*-methylmannose run as controls. It gave mannose on demethylation.

Fraction 7 (1037—1365 c.c.). A syrup (27 mg.), $[\alpha]_D$ +52° (*c* 0·9 in acetone) R_G 0·56 (Found: OMe, 29·2. Calc. for $C_8H_{16}O_6$: OMe, 29·6%), gave only glucose on demethylation and was chromatographically identical with 2 : 3-di-*O*-methylglucose run as a control (solvents 6 and 7). The derived aniline derivative had m. p. 133°, undepressed on admixture with specimen kindly given by Professor M. Stacey, F.R.S.

Fraction 8 (aqueous eluant). An amorphous solid (12·3 mg.), $[\alpha]_D$ −9° (*c* 0·3). Deionisation [IR-120(H^+] resin) gave an acidic syrup which revealed spots of R_F 0·70, 0·45, 0·40, 0·34, and 0·15 respectively (paper chromatography, solvent 5).

Polysaccharide (*B*).—The chloroform-insoluble acetate (B) had $[\alpha]_D$ +38° (*c* 1·15 in C_5H_5N) (Found: Ac, 22·0; SO_4^{2-}, 5·7%). Determination of the molar proportions [3] of the sugars present in a hydrolysate gave galactose : glucose : xylose : rhamnose = 0·06 : 0·56 : 1·69 : 1·31. Deacetylation and methylation of the residual material (11·3 g.) as for polysaccharide (A) gave a methylated product (5·3 g.), $[\alpha]_D$ −28° (*c* 1·3 in $CHCl_3$) (Found: OMe, 35·1; SO_4^{2-}, 5·4%). Further Purdie methylations failed to raise the methoxyl content. Fractionation of the methylated material (5·12 g.) by treatment with light petroleum (b. p. 60—80°) and addition of increasing quantities of chloroform gave the following fractions. Complete dissolution occurred in a mixture containing 40% chloroform (v/v) :

Fraction	1	2	3	4	5	6	7	8
$CHCl_3$ in mixture (%)	0	5	10	15	20	25	30	40
Weight (mg.)	24	47	106	386	635	972	943	2060

A sample of each fraction was hydrolysed (N-H_2SO_4, 7 hr., 100°), and the hydrolysate examined by paper chromatography. Each of the hydrolysates contained methylated uronic acids (solvent 3) and exhibited no appreciable quantitative difference in the neutral methylated sugars present. The fractions were combined (5·1 g.) and hydrolysed as for the methylated polysaccharide (A) except that hydrolysis with aqueous acid was continued for 18 hr. The product (4·1 g.) was separated on a cellulose column (52 × 3 cm.) with water-saturated light petroleum–butan-1-ol (7 : 3); R_G values are recorded below for solvent (6).

Fraction 1. 2 : 3 : 4-Tri-*O*-methylxylose (87 mg.), R_G 0·95, $[\alpha]_D$ +22° (*c* 0·8) (Found: OMe, 48·0. Calc. for $C_8H_{16}O_5$: OMe, 48·1%). Demethylation afforded xylose. The aniline derivative had m. p. and mixed m. p. 97°.

Fraction 2. Crystallised slowly from methanol (230 mg.), this had m. p. 99°, R_G 0·88, M_G 0·0 (cf. 3 : 4-di-*O*-methylrhamnose M_G 0·36), $[\alpha]_D$ +42° (*c* 1·5) (Found: OMe, 32·4. Calc. for $C_8H_{16}O_5$: OMe, 32·3%). Demethylation gave rhamnose. The reduction of sodium periodate by this fraction and by 2 : 4-di-*O*-methylrhamnose was measured.[12] This was constant with both sugars after 20 hr. and corresponded to 1·6 and 0·9 mol. of periodate respectively. After addition of ethylene glycol the oxidation mixtures were examined by paper chromatography [13] (solvent 6). The product from fraction (2) gave one main spot, of R_G 0·81, whereas that from 2 : 4-di-*O*-methylrhamnose gave spots of R_G 0·72, 0·78, and 0·85 severally (aniline oxalate spray). *X*-Ray powder photographs of the crystalline sugar and of authentic 2 : 4-di-*O*-methylrhamnose were different.

Fraction 3. 2 : 3 : 6-Tri-*O*-methylglucose (35 mg.), m. p. and mixed m. p. 122°, R_G 0·81, $[\alpha]_D$ +70° (*c* 0·95) (Found: OMe, 42·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Demethylation gave glucose.

Fraction 4. Syrupy 2 : 3-di-*O*-methylxylose (250 mg.), R_G 0·77 (cf. 2 : 4-di-*O*-methylxylose R_G 0·66), $[\alpha]_D$ +21° (*c* 3·0) (Found: OMe, 34·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). Demethylation gave xylose. The aniline derivative had m. p. and mixed m. p. 145°.

Fraction 5. Syrupy 2-*O*-methylrhamnose (464 mg.), R_G 0·60, M_G 0·06 (cf. 3-*O*- and 4-*O*-methylrhamnose M_G 0·37 and 0·48 respectively), $[\alpha]_D$ +37° (*c* 3·5). The derived methylglycopyranoside reduced 1·1 moles of sodium metaperiodate per mole of glycoside. Paper chromatography of the oxidation products of the free sugar revealed a single spot of R_F 0·78. 4-*O*-Methylrhamnose on similar treatment gave a spot of R_F 0·95 (solvent 6). The aniline derivative had m. p. 146° undepressed on admixture with an authentic specimen of 2-*O*-methyl-*N*-phenylrhamnosylamine kindly supplied by Dr. L. Hough.

Fraction 6. Syrupy 2 : 3-di-*O*-methylglucose (14 mg.), R_G 0·52 (Found: OMe, 29·5. Calc. for $C_8H_{16}O_6$: OMe, 29·6%). Demethylation gave glucose. The aniline derivative had m. p. and mixed m. p. 132° [cf. polysaccharide (A), fraction 7].

Fraction 7. A syrup (26 mg.), R_G 0·30; in solvent (5), R_G 0·75.

Fraction 8, aqueous eluant. An amorphous solid (2·06 g.) which after treatment with IR-120(H^+) resin gave an acidic syrup (1·61 g.). Chromatography in solvent (5) gave spots of R_F 0·72, 0·53, 0·33, 0·25, 0·13, and 0·10; and R_G values of 0·87, 0·66, 0·41, 0·30, 0·16, and 0·12 respectively. Attempted separation on a cellulose column was unsuccessful, but was achieved on thick paper with this eluant: six fractions were collected.

The first (i), a syrup (161 mg.), R_G 0·87, $[\alpha]_D$ −12° (*c* 1·6), was converted into the ester glycoside and reduced with lithium aluminium hydride as for the aldobiuronic acid. This product was hydrolysed with N-hydrochloric acid at 100° for 4 hr. The resultant syrup (95 mg.) revealed two main spots, of R_G 0·84 and 0·61 respectively, together with a faint spot of R_G 1·15 (paper chromatography, solvent 6). Separation on thick paper (3MM) afforded: 2 : 3 : 4-tri-*O*-methylglucose (38 mg.) (characterised as below) and 2-*O*-methylrhamnose (36 mg.), R_G 0·61, $[\alpha]_D$ +34° (*c* 1·8), chromatographically and ionophoretically identical with fraction 5 separated from the hydrolysate of the neutral sugars from the methylated polysaccharide (B). After periodate oxidation this substance revealed a single spot, R_F 0·78 (solvent 6).

The other fractions (ii, 56 mg.; iii, 154 mg.; iv, 117 mg.; v, 360 mg.; and vi, 207 mg.) were each converted into their ester glycoside, reduced, and hydrolysed. Chromatography (solvent 6) of each hydrolysate revealed that fractions ii—v each gave two spots, of R_G 1·15 and 0·84. Fraction ii also gave a faint spot of R_G 0·61. Fraction vi gave the same spots as fractions ii—v and in addition spots of R_G 0·48, 0·33, 0·18, and 0·10 severally. Yields were as tabulated. Fractions ii—v were combined and separated into two further fractions (*a* and *b*) on thick paper.

Fraction	ii	iii	iv	v	vi
Weight (mg.)	56	154	117	360	207
Yield after reduction	31	92	68	204	82

(*a*) 2 : 3 : 4-Tri-*O*-methylglucose (142 mg. + 38 mg. from fraction i), R_G 0·84, $[\alpha]_D$ +53° (*c* 0·66) (Found: OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%), gave an infrared spectrum identical with that from 2 : 3 : 4-tri-*O*-methylglucose. The aniline derivative had m. p. 131°, not depressed on admixture with 2 : 3 : 4-tri-*O*-methyl-*N*-phenylglucosylamine, m. p. 139°.

(*b*) A yellow non-reducing syrup (113 mg.), R_G 1·15 (solvent 6), $[\alpha]_D$ +41° (*c* 2·0), was unattacked by periodate and gave a positive test for methoxyl. Comparison of the infrared spectra of this material with that of reduced (lithium aluminium hydride) 2-*O*-methylrhamnose revealed that, while not identical in the finger-print region, the spectra indicated similarity in the molecular structure.

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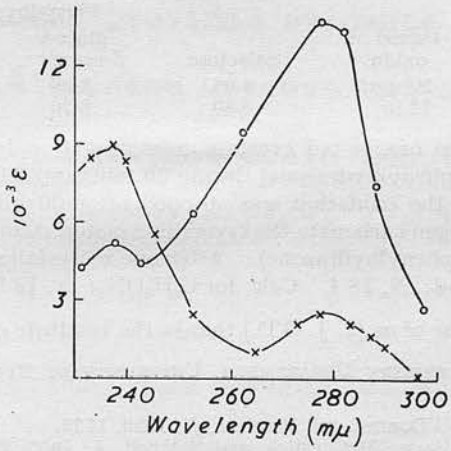
462. *Ultraviolet Spectra and Periodate Oxidation of Monosaccharide Phenylhydrazones.*

By J. J. O'Donnell and Elizabeth Percival.

In work on the repeated Barry degradation of laminarin and cladophoran [1] it became apparent that the periodate ion attacked the nitrogen-containing residues present in the degraded polysaccharides, the nitrogen content being reduced on subsequent oxidation. To obtain information on the nature of these nitrogen-containing residues and quantitative values for the amount of periodate reduced by these groups in the degraded polysaccharide the ultraviolet absorption spectra of, and the action of periodate on, sugar phenylhydrazones have been studied. It was expected that the uptake of periodate by such compounds might be related to that of the degraded polymers.

The ultraviolet spectra of sugar osazones [2] and of the Barry degradation fragments of monomethyl-sugars [3] have already been examined. The spectra of the sugar phenylhydrazones exhibited qualitative and quantitative similarities, but these could not be related to the spectra of the degraded polysaccharides. When the solutions were kept the phenylhydrazone spectra changed to a non-characteristic curve, in keeping with the tendency of these compounds to decompose in solution.



Ultraviolet absorption of (○) monosaccharide phenylhydrazones and (×) the Skraup form of glucose phenylhydrazone.

Oxidation of sugar osazones [4] by sodium periodate resulted in rapid consumption of the theoretical amount of periodate, and precipitation of mesoxaldehyde 1 : 2-bisphenylhydrazone; this was followed by a slow irregular reduction which was attributed to interaction of the periodate ion with the insoluble mesoxaldehyde derivative. A similar reaction takes place with the phenylhydrazones, and orange-red crystals believed to be glyoxal monophenylhydrazone are deposited. The initial rapid consumption of periodate was complete within an hour and corresponded to the number of glycol groups. This was followed by a further uptake of 1·6—2·8 mol. during 3 days. These results, together with the earlier results on periodate oxidation of osazones, demonstrate clearly that the periodate ion attacks, not only the glycol groups, but also the nitrogenous group in the degraded polymers. Since oxidation of degraded nitrogen-containing polysaccharides is usually allowed to proceed for several days the extent of this attack is considerable and any quantitative conclusions based on it must be viewed with caution.

In this work it was found that, whereas the β-form of glucose phenylhydrazone had adsorption characteristics similar to those of the other sugar phenylhydrazones, the Skraup isomer [4] of glucose phenylhydrazone gave a different spectrum (see Figure) which was

qualitatively similar to that of phenylhydrazine. This agrees with the results of Mester and Major [5] who postulated, on the basis of the formation of a crystalline formazan, that glucose β-phenylhydrazone (like mannose phenylhydrazone) had an acyclic structure. In contrast, Skraup's glucose phenylhydrazone did not yield a formazan and was therefore considered to have a cyclic structure.

Experimental.—The phenylhydrazones of mannose, ribose, arabinose, galactose, and glucose (β-form and Skraup form), prepared by the standard method,[6] were isolated as white crystalline solids and recrystallised to constant m. p. The absorption of each phenylhydrazone (10 mg./l.) in 1 : 1 aqueous ethanol was measured in a Unicam S.P. 500 spectrophotometer in the range 222—230 mμ. The value of ε at 277 mμ for each sugar was constant (±10%) at 13,700. A second less well-defined peak at 235—240 mμ was also present. The Skraup isomer had a negligible absorption at 277 mμ but at 237 mμ had ε 8900 (cf. phenylhydrazine which also has a maximum absorption at this wavelength). Degraded laminarin and degraded cladophoran (142 mg./l.) showed weak absorption peaks at 383—385 and 360—362 mμ respectively.

Periodate oxidation. Samples (100 mg.) were dissolved in water (5 ml.) (mannose phenylhydrazone gave a suspension), and 0·197M-sodium periodate solution) (15 ml.) was added. The reaction was followed by withdrawal of samples at intervals, and the amount of periodate reduced was measured.[7] The quantity of periodate (3 or 4 mols.) required to cleave the glycol groups present in each hydrazone was consumed within 1 hr. and this was accompanied by the precipitation of orange-red crystals. As oxidation proceeded these crystals gradually redissolved and a tar resulted; this had no distinguishing feature in its ultraviolet spectrum. The periodate consumptions (mole/mole) were:

	Phenylhydrazones of:				
Period of oxidn.	galactose	glucose β-isomer	glucose Skraup isomer	arabinose	ribose
30 min.	4·05	3·90	3·95	3·06	3·16
72 hr.	6·30	6·20	6·80	4·60	4·90

The orange-red crystals consumed 1 mole of periodate per mole (calculated as glyoxal monophenylhydrazone) during 30 min. and 2·6 moles per mole during 48 hr.

If the oxidation was stopped after 30 min. by addition of ethylene glycol and sodium hydrogen carbonate the crystalline material could be separated (80% yield calculated as glyoxal monophenylhydrazone). After one recrystallisation from alcohol this had m. p. 85° (decomp.) (Found: N, 18·4. Calc. for $C_8H_8ON_2$: N, 18·9%).

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Polysaccharides from the Green Seaweed *Caulerpa filiformis* Part II.[†] An Amylopectin-type Glucan.

By I.M.Mackie and Elizabeth Percival

Addition of cetyl trimethylammoniumhydroxide to the borate complexes of the mixture of water-soluble polysaccharides isolated from *Caulerpa filiformis* led to the separation of a pure glucan. Methylation and periodate oxidation studies provided evidence that this glucan contains α-1,4'-linked glucose units with branch points at C_6 and has an average chain length of *ca* 24. The results of enzymic investigations are in harmony with this structure.

A starch-type polysaccharide, the so-called Floridean starch[1], has been isolated from the red seaweed, Dilsea edulis, and an amylopectin-type polysaccharide has been obtained from the blue green fresh water alga, Oscillatoria[2]. In the field of the green seaweeds, however, although it is often accepted that the reserve carbohydrate is a starch, there is no record of the isolation and characterisation of a starch-type polysaccharide.

[†] Part I, Mackie and Percival, J., 1959, 1151

Hitherto fractionation of the complex mixture of water-soluble polysaccharides isolated from these seaweeds has not been achieved. O'Donnell and Percival[3] reported the separation of a glucose-rich fraction from the mixture of acetylated polysaccharides extracted from Acrosiphonia centralis, and characterised this glucose containing material as a starch-like polymer containing α-1,4'-linked glucose units with branch points at C_6. The present paper describes an investigation of a glucan fractionated from the mixture of polysaccharides extracted by dilute acid from Caulerpa filiformis.

The water-soluble sulphated polysaccharides, $[\alpha]_D$ +120° extracted from Caulerpa filiformis (see Part I), was treated in the form of their borate complexes with an aqueous solution of cetyl trimethylammoniumhydroxide. This precipitated sulphated polymeric material and left in the supernatant liquid a pure glucan which was precipitated as an amorphous powder with ethanol. It contained 98% glucose and 0.9% ash and had a limiting viscosity number $[\eta]$, measured in 0.1M sodium chloride solution, of 15. An aqueous solution, which had $[\alpha]_D$ +154°, gave a purple colour with iodine, exhibiting maximum absorption at 540 mμ. On treatment with salivary α-amylase it gave an apparent percentage conversion into maltose of 90. The β-amylolysis limit was 57 and this was increased to 83 after treatment with isoamylase. Attempted fractionation by

the thymol method[4] in the absence of oxygen failed to separate any amylose. The periodate consumption by this polysaccharide corresponded to ca 1 mole for every anhydro-sugar residue, and the oxopolysaccharide, isolated after dialysis, was devoid of unattacked glucose units. This provides qualitative evidence that 1,2- and 1,3-glucosidic linkages are absent and that the polysaccharide contains 1,4-linked glucose units with possible branch points at C_6. The production of formic acid on oxidation at room temperature with potassium metaperiodate[5] corresponded to an average chain length of 21.

The properties of this polysaccharide are compared with other branched 1,4-linked glucans in the table below:

Comparison of the Properties of Amylopectin, Floridean starch, Glycogen and the Amylopectin-type Glucan

Property	Amylopectin[6]	Floridean starch[6]	Glycogen[6]	Glucan
$[\alpha]_D$ in H_2O	+212°	+176°	+196	+154°
Iodine Colouration	Purple	Deep red-dish brown	Reddish brown	Purple
λmax of absorption spectrum of iodine complex	540	500	460	540
Optical density at λ max	1.06	0.84	0.34	0.68
β-Amylolysis limit	54	46	45	57
Iso- and β-amylolysis limit	76	54	65	83
α-Amylolysis (% P_M+)	88	65	70	90
Reduction of IO_4 moles/ anhydro-glucose unit	1.04	1.05	1.08	0.95
Average chain length	20	9	12	21
Limiting viscosity number	ca 150	-	10	15

+P_M = Apparent conversion into maltose

This comparison reveals that the glucan from C. filiformis and the amylopectin component of the starch of land plants have many properties in common.

Methylation of the glucan was carried out with sodium and methyl iodide in liquid ammonia[7]. The low yield (ca 50%) of partially methylated glucan isolated after a single methylation is probably due to loss of shorter-chain material during dialysis of the methylated material. The loss of staining power to iodine caused by methylation of the glucan is further proof of an amylopectin-type structure since Hirst, Jones and Roudier[5] record that methylated amylopectins give no appreciable colour with iodine.

The tri-O-methyl derivative, $[\alpha]_D$ +200°, had a number average molecular weight of 15,120, corresponding to 76 anhydro-glucose units, when measured by the isothermal distillation method[8]. Although this value is small for an amylopectin-type polysaccharide, the method of extraction, dilute acid (pH 3-4) at 70° for 6 hours followed by deproteinisation with trichloracetic acid, would undoubtedly degrade the polysaccharide considerably, and there is no doubt that in the native state the glucan has a much larger molecule.

The tri-O-methyl derivative was heated with methanolic hydrogen chloride and the methylated methyl glucosides so obtained were hydrolysed with aqueous hydrochloric acid.

The mixture of reducing sugars were separated on a column of powdered cellulose into 2,3,4-tetra-O-methylglucose (ca. 1 part), 2,3,6-tri-O-methylglucose (ca. 22 parts), 2,3-di-O-methylglucose (ca. 1 part) which probably owed their origin to demethylation during hydrolysis. A trace of monomethylglucose, and no free glucose, was found. These results confirm the presence of α-1,4'-linked glucose residues with probable branch points at C_6; the α-linkage being inferred from the high positive rotations of the glucan and its trimethyl ether. The molecular proportion of tetra-O-methylglucose isolated corresponds to the presence of one non-reducing terminal glucose residue to every 28 glucose units in the molecule. This value, which is higher than the value of 21 found from periodate oxidation experiments on the unmethylated material, is in agreement with the postulated loss of shorter-chain material during dialysis of the partially methylated polysaccharide.

Experimental

The analytical methods used have been described by O'Donnell and Percival[3], the solvent system number (6) being used for paper partition chromatography.

Separation of the Glucan.- To the water soluble polysaccharide material (12 g.), isolated from Caulerpa filiformis (Part I), dissolved in water (1 l.) 0.6 M-boric acid (400 c.c.) was added with stirring. The further addition of an aqueous solution of 0.1N-cetyl trimethylammonium hydroxide (CTA-OH) (180 c.c.) and 0.5N-sodium hydroxide (4.0 c.c.)[9] produced a flocculent precipitate (A). After removal of (A) a further addition of CTA-OH yielded a negligible amount of precipitate. Addition of ethanol to the supernatant gave a gelatinous precipitate (B).

Properties of the Glucan.- Adhering reagent was removed from (B) by thorough washing with ethanol and ether. The resulting white amorphous powder (3.9 g.) was soluble in cold water, had $[\alpha]_D$ +154° (c 1.0) (Found: Ash, 0.9%; SO_4^{2-}, nil; N, nil). An acid hydrolysate (N-H_2SO_4 at 100°, 4 hr.) contained only glucose (paper chromatography) (Found: glucose by cuprimetric titration 98.0%) which was separated as crystals [m.p. and mixed m.p. with glucose hydrate 82°, $[\alpha]_D$ +52.0° (c 1.0)] in 77% yield. The polysaccharide gave a purple colour with iodine exhibiting maximum absorption at 540 mμ.

Viscosity Determination.- The specific viscosity (η_{sp}) of 0.1 M-sodium chloride solutions of the polysaccharide was determined at several concentrations at 25°, and the limiting viscosity number $[\eta]$ determined graphically from the relation $[\eta] = \lim_{c \to 0} (n_{sp/c})^{10}$. Solvent time = 647.33 (T_o). Final concentrations (c) are expressed as g. per ml. solution.

	T sec	$\frac{T - T_o}{T}$	$n_{sp/c}$	c
15 ml.	738.0	0.1401	15.66	0.00895
" + 5 ml.0.1M-NaCl	714.18	0.1032	15.38	0.00671
" + " "	700.14	0.0816	15.20	0.00537
" + " "	691.50	0.0683	15.30	0.00447

From graph of $n_{sp/c}$ against c x 10^3 $[\eta]$ = 15

Enzymic Degradation.- (a) Salivary α-amylase. Polysaccharide (26.2 mg.), sodium chloride (5 mg.) and freeze-dried salivary α-amylase (5 mg.) kindly supplied by Dr. D.J.Manners) in a total volume of 50 c.c. was incubated at 35° for 48 hr. The P_M value was 90. In a control experiment, potato starch (25.8 mg.) gave a P_M value of 88.

(b) Soya-bean β-amylase (with Dr. D.J.Manners and Mr. A. Wright). Polysaccharide (12.8 mg.) was incubated with 0.2 M-acetate buffer (pH 4.6, 3 c.c.) containing soya-bean β-amylase solution (0.05 c.c.; 1000 units) in a total volume of 25 c.c. after 48 hr. the β-amylolysis limit was 57%.

(c) Iso-amylase and β-amylase (with Dr. D.J.Manners and Mr. A. Wright). Polysaccharide (20 mg.) in acetate buffer (pH 5.9, 6 c.c.) and water (5 c.c.) was treated with isoamylase solution (50 mg.) at room temperature for 65 hr. (the isoamylase was extracted from brewer's yeast by Miss Zeenat H. Gunja) After inactivation of the isoamylase by heat denatured protein was centrifuged off. To the supernatant solution (10 c.c.), 0.02M acetate buffer pH 4.6 (5 c.c.) and β-amylase (20 units per mg. polysaccharide) and water (to 25.0 c.c.) were added.

Periodate Oxidation.- (i) Uptake of Periodate. The dry glucan (447 mg.) was oxidised with 3% sodium periodate and the reduction of periodate was measured at intervals[12].

Time of Oxid^{n} (hr.	1	3.5	4.5	24	76	96	120
Moles $NaIO_4$ consumed/ $C_6H_{10}O_5$	0.739	0.848	0.864	0.933	0.957	0.960	0.980

Chromatographic examination of the hydrolysis solution of the oxopolysaccharide (isolated after 120 hr. oxidation) showed the absence of glucose.

(ii) Potassium Metaperiodate Oxidation. Glucan (159 mg.) dissolved in 3% potassium chloride solution (80 c.c.) was oxidised with 4% sodium metaperiodate solution (80 c.c.) was oxidised with 4% sodium metaperiodate solution (20 c.c.) at room temperature in the dark[5]. Portions (10 c.c.) were analysed at intervals:

Time of Oxid^{n} (hr.)	16	64	88	160	208	256
Moles x 10^{-2} HCOOH/ $C_6H_{10}O_5$	0.91	3.1	3.8	4.2	4.7	4.7
Apparent $\overline{CL}$ (Glucose residues)						21

Methylation in Liquid Ammonia.- The dried glucan (3.0 g.) was methylated in liquid ammonia with sodium and methyl iodide under the conditions used by Mackie and Percival (Part I). After addition of water the aqueous mixture was dialysed until free from inorganic ions. The partially methylated polysaccharide was isolated by freeze-drying and subjected to two further methylations under the same conditions. The product (1.4 g.) had $[\alpha]_D^{17}$ +200° (c 1.0 in $CHCl_3$) (Found: OMe, 45.3. Calc. for a tri-O-methylglucan: OMe, 45.6%). It was soluble in water, ethanol, chloroform and benzene, and gave no colouration with iodine.

Determination of the Degree of Polymerisation by Isothermal Distillation[8]. The methylated glucan (73.4 mg.) (after drying to constant weight; 24 hr. at 80°/12 mm. over P_2O_5) was dissolved in dried analar benzene (6.953 g.); the solution having a concentration of 1.05%. The changes in level of solution and solvent, in an apparatus kindly lent by Dr. T.G.Greenwood, were measured over a period of 100 hr. The graph of change in level with time was a straight line and the slope of the graph was the rate of distillation, which is proportional to the solute mole

fraction The apparatus constant K (2.2×10^{-3}) was determined by measuring the rate of distillation for benzene with mole fractions of triolein (Mol. wt. 885.4). The value of K was the same whether calculated for the change in solvent level or for the change in solution level. The slope of the graph was 0.237/95 mm./hr., and the calculated number average molecular weight 15,120.

Hydrolysis of the Methylated Polysaccharide.- The material (1.1 g.) was refluxed with methanolic hydrogen chloride (50 c.c., 3%) until the rotation was constant (7 hr.). Water (150 c.c.) was added and, after removal of methanol under reduced pressure, the mixture was heated at 100° until the rotation was again constant (6 hr.). After neutralisation (Ag_2CO_3), deionisation with hydrogen sulphide and Amberlite resins, and concentration, the resulting syrup (0.9 g.) was separated into its constituents on a cellulose column under the conditions used for the methylated xylan hydrolysate (Part I). The fractions were weighed after filtration through "Filter Cel", concentration to dryness, dissolution in methanol, filtration, and concentration.

Fraction I. Crystalline material (95.7 mg.), R_G 1.0 which had m.p. 40°, $[\alpha]_D$ -16° was apparently a mixture of tetra-O-methylglucose and methyl 2,3,6-tri-O-methylglucoside. It was therefore heated with N-hydrochloric acid (5 c.c.)

at 100° until the rotation was constant (14 hr.) After neutralisation, deionisation and concentration a syrup was obtained which was separated into fractions Ia and Ib on a cellulose column (30 x 1.5 cm.).

Fraction Ia. Crystalline 2,3,4,6-tetra-O-methylglucose (25 mg.), R_G 1.0, m.p. and mixed m.p. 84° after recrystallisation from ether, $[\alpha]_D$ +80° (c 2.4). The derived anilide had m.p. and mixed m.p. 135°.

Fraction Ib. Crystalline 2,3,6-tri-O-methylglucose (70 mg.), R_G 0.83, had m.p. and mixed m.p. 115° after recrystallisation from ether (Found: OMe, 41.0. Calc. for $C_9H_{18}O_6$: OMe, 41.9%).

Fraction II.- Syrupy methyl 2,3,6-tri-O-methylglucoside, after heating with N-hydrochloric acid (5 c.c.) at 100° for 14 hrs. and neutralisation, gave syrupy 2,3,6-tri-O-methylglucose (228 mg.), R_G 0.83, $[\alpha]_D$ +70° (c 2.3).

Fraction III.- Crystalline 2,3,6-tri-O-methylglucose (259 mg.), R_G 0.83, had m.p. and mixed m.p. 115° after recrystallisation from ether, $[\alpha]_D$ +98° (Initial) → +70° (const.)(c 2.5); $[\alpha]_D$ +70° (initial), dropping to -35° (after 10 hr. in 1%MeOH-HCl at 18°; c 1.0) (Found: OMe, 41.1%). Total yield of 2,3,6-tri-O-methylglucose, 557 mg.

Fraction IV.- Syrup (19.5 mg.), M_G 0.10, R_G 0.54 and 0.83 (trace), $[\alpha]_D$ +53° (c 2.0).

Fraction V.- Syrupy 2,3-di-O-methylglucose (8.0 mg.).

The M_G (0.10) and R_G (0.54) values were identical with those of 2,3-di-O-methylglucose, $[\alpha]_D$ +48°. The derived anilide had m.p. and mixed m.p. 132°.

Fraction VI.- Syrup (34 mg.), $[\alpha]_D$ +42°, R_G 0.51. The M_G (0.05 and 0.65) values were identical with those of 2,6- and 3,6-di-O-methylglucose respectively.

Fraction VII.- Syrup (8.3 mg.), R_G 0.25 corresponding to mono-O-methylglucose. Aqueous washing of the column failed to yield any further carbohydrate.

Examination of the Precipitated Sulphated Polysaccharides.- To regenerate the polysaccharides, the precipitate (A) after thorough washing with ethanol was dissolved in warm 1M-sodium chloride solution and the mixture poured into ethanol. The precipitate (C) was obtained as a white powder after filtering and drying. Hydrolysis of a portion with N-sulphuric acid at 100° for 7 hr. and chromatographic analysis of the resulting syrup showed the presence of galactose, glucose, mannose, xylose and rhamnose. Incubation of the polysaccharides (C) with salivary α-amylase, followed by dialysis and precipitation with ethanol gave material which was devoid of glucose. Fractionation experiments on this glucose-free sulphated material are in progress and will form the subject of a future communication.

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